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EFFECTS OF INBREEDING ON VARIABILITY [1] IN ALFALFA

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INTRODUCTION

Until recently alfalfa was regarded as a plant that was freely cross-fertilized, presumably by certain large bees, thereby implying a high degree of heterozygosity. It was thought to behave in a manner somewhat similar to corn, though probably in a lesser degree. Corn is so highly heterozygous that it is only after several generations of inbreeding that strains approaching uniformity are obtained. However, field observations have thrown doubt on the necessity of the bees, and Carlson's [3] tripping studies indicate the likelihood of considerable self-fertilization in alfalfa. The present paper, which shows that many strains of alfalfa become almost uniform as a result of one generation of inbreeding, makes it clear that alfalfa is much less heterozygous than was formerly supposed.

MATERIAL AND METHODS

Seeds of about a dozen standard varieties of alfalfa, *Medicago sativa* L., largely furnished by the Division of Forage Crops and Diseases, United States Department of Agriculture, were sown in hills at the Uintah Basin alfalfa-seed experimental farm near Fort Duchesne, Utah, in the spring of 1926 and 1927. An alfalfa variety plot had previously been kept at the college farm at Logan, Utah, but since alfalfa set seed only occasionally at Logan, selections made from this plot were added to the nursery at Fort Duchesne. Several previously close-bred selections were also obtained from L. E. Kirk, then of the Saskatchewan Experimental Station in Canada. In 1928 a large number of individual plants of the following varieties from these hills were self-fertilized: Utah Common, Grimm, Saskatchewan No. 666 (selections from Grimm furnished by Kirk), Hardigan, Dakota Common, and Ontario Variegated. Self-fertilization was accomplished by bagging branches with 2-pound kraft grocery bags, previously determined to be the best type of bag for this purpose. A branch of a large alfalfa stem was selected on which the blossoms were well developed but not yet stripped. All blossom clusters showing any evidence that flowers had been exposed to pollination were clipped off. The paper bag was then securely tied with a cotton

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[2] Appreciation is expressed to J. W. Carlson, superintendent of the Uintah Basin substation, for help in growing the plants and in taking field data; to Prof. Calvin Fletcher, head of the Art Department, Utah State Agricultural College, for assistance in determining the numerical color values to be assigned to various blossom and foliage colors, to Wesley Keller, technician, Intermountain Forest and Range Experiment Station, Forest Service, and to Dr. F. V. Owen, geneticist, Division of Sugar Plant Investigations, U.S. Department of Agriculture, for reading the manuscript and for checking all calculations. Dr. H. K. Hayes, chief of the Department of Agronomy and Plant Genetics, Minnesota Agricultural Experiment Station, kindly read a preliminary draft of the manuscript and made several valuable suggestions.

[3] CARLSON, J. W. ARTIFICIAL TRIPPING OF FLOWERS IN ALFALFA IN RELATION TO SEED PRODUCTION. Jour. Amer. Soc. Agron., 22:780-786. 1930.

string below the blossom cluster (fig. 2, *B*). Ordinarily, 10 bags were placed on each plant, care being taken to choose branches that were easily traced to a single root. Self-fertilized seeds were obtained from about 160 plants. The number of seeds obtained from each plant varied from 4 or 5 to several hundred.

In 1929 these seeds were sown in 50-hill progeny rows, provided there were enough seeds; otherwise, 20-hill rows were seeded except in a few cases where there were seeds enough for only 10-hill rows. In all, there were 150 progenies. Of these, 84 were in 50-hill rows, adjacent to which was a single 50-hill row of each parental variety of the same age. With careful irrigation, good stands were secured and all hills were carefully thinned to a single plant in those progenies of which enough seeds had been available to sow more than one seed in a hill. There were some missing hills, but in nearly all of the 50-hill rows 30 or more plants were obtained, the number in the shorter rows varying from 20 downward. A few rows failed entirely and several had only 3 or 4 plants. After eliminating the rows with less than 7 plants, the number of progenies by varieties was as follows: Utah Common, 30; Grimm, 14; Saskatchewan No. 666 (Grimm), 30; Hardigan, 12; Dakota Common, 9; and Ontario Variegated, 6; this represented a total of 101 first-generation inbred progenies.

In 1930 when the plants had reached about the half-bloom stage, observations were made of various plant characters. The most striking characteristic exhibited was the high degree of uniformity in many progenies as to height and erectness of plant, thickness of stem, leaflet size, and color of leaves and blossoms. More than half the progenies appeared rather uniform for one or more visible plant characters and many of them for two or more. This result was so marked as to suggest a study of variability, the data for which were taken during the next few days, regarding (1) plant height, (2) plant width, (3) angle of erectness (from the ground), (4) diameter of stem, (5) leaflet length, (6) leaflet width, (7) color of blossom, and (8) color of leaflet. A freak blossom, a stem coloration, and some plant peculiarities were also noted.

Height and width of plant were measured in inches and the angle of erectness in degrees by means of a large protractorlike diagram drawn on heavy cardboard. Diameter of stem was obtained about 3 inches above the ground on a leading stem of each plant to the nearest one-tenth millimeter by means of calipers. Leaflet length and width were read to the nearest millimeter on a small celluloid ruler. Blossom color and leaf color were determined by matching with Ridgway's [4] Color Standards, the number of the color being recorded for each plant. This color determination required considerable time but was done with the utmost care. A single row of each parental variety which had been seeded adjacent to the progenies was studied in the same way to obtain check data. The data were then studied and the mean of each character calculated for each progeny, together with standard deviations and coefficients of variability.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

EXPERIMENTAL DATA AND THEIR INTERPRETATION

HEIGHT OF PLANT

Data regarding plant height are presented in table 1, where the progenies of 7 to 40 plants each and the respective parental variety of 35 to 38 plants each are arranged according to plant-height and coefficient-of-variability classes. The entire progeny or row is regarded as a unit.

In all of the six varieties studied, there are some progenies much less variable than the parent rows, some about equally variable, and a few apparently more variable. The parent stock of Utah Common showed a variability of 21.8 percent, whereas several of the inbred progenies had coefficients of variability of 15 percent or less, one being as low as 9.5 percent.

TABLE 1.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to plant-height classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*

C.V. class (percent)	Number of progenies in indicated plant-height class, in inches									
	14	16	18	20	22	24	26	28	30	32
UTAH COMMON										
9					1					
12						2	1	1		
15					1	2	2			
18				2		2				
21				1	1	3	*x*			
24				2	2	3				
27			1	1						
30						1				
33										
36										
39			1							
GRIMM										
12						1				
15					1	1				
18					2	1		1		
21	1		1	1		1				
24				1	[1] 1*x*					
27										
30										
33										
36			1							
SASKATCHEWAN NO. 666										
9							2	1		
12				2						
15				3						
18					1	1				
21				3	3	1				
24		1	2	1	2	2				
27		1	1*x*	1	1	1				
30										

[1] 1*x*=1 progeny in same class as parental row; 2*x*=2 progenies in same class as parent.

TABLE 1.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to plant-height classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*—Continued

C.V. class (percent)	Number of progenies in indicated plant-height class, in inches									
	14	16	18	20	22	24	26	28	30	32
HARDIGAN										
9						1				
12					1	2				
15						1	1			
18					1					
21					1*x*	2				
24										
27			1				1			
30										
DAKOTA COMMON										
12							2			
15					2	1				
18							2			
21							1		*x*	
24										
27			1							
ONTARIO VARIEGATED										
9								1		
12										
15						1				
18						3				
21					1					
24						*x*				

Grimm had a coefficient of variability of 22.9 percent. Seven of fourteen progenies had coefficients of 18 percent or less and one was 12.2 percent. As might be expected, Saskatchewan No. 666, previously close-bred, showed even more uniformity, 3 progenies having a coefficient of variability as low as 9 percent and 7 others between 12 and 18 percent, as compared to 27 percent for the parental check row.

The parental varieties Hardigan, Dakota Common, and Ontario Variegated had coefficients of variability of 22, 21.9, and 25 percent, respectively, whereas about one-third to one-fourth of the entire number of progenies of each variety had coefficients of 9 to 12 percent.

In order to determine whether some inbred progenies were low in variability, the significance of the differences in variability between the parental variety (check) and two inbred progenies of the same variety was studied both by the probable-error method and by Fisher's z test.[5] In using the z test it was considered advisable to make a slight departure from Fisher by employing the coefficient of variability rather than the standard deviation since in some instances the means varied widely; in Grimm progenies, for example, the plant-height classes ranged from 14 to 28 inches. The coefficient of variability was also better suited to the variability studies presented in the odd-numbered tables between tables 1 to 11 and in tables 14 and 16. The first progeny studied is the least variable one, or from the least variable class. The second progeny is a more or less random selection

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3 rev. and enl., 283 pp., illus. Edinburgh and London. 1930.

from the group in the next higher variability class. Thus, 2 variability classes are always given for each inbred variety, and not merely 2 progenies. Saskatchewan No. 666, for example, had 3 progenies in the same variability group as the least variable progeny and 2 in the second group. Only 2 progenies are studied, but 5 have a variability approximately equal to those studied. In the entire table, 23 progenies out of 101 have very low variability, which is about 23 times what would be expected from chance alone. Throughout this paper, each progeny studied represents two groups varying from 1 to several progenies having approximately equal variability, the total usually being 15 to 20 for the entire 101 progenies. The difference in coefficients of variability is obtained by subtracting the coefficient of variability of each progeny from that of its check row and calculating the error of the difference and the odds according to Fisher to see whether the variability is significantly less in the inbred progenies. This difference in coefficients of variability divided by the error of the difference (Diff./P.E.) ranged for plant height, from 3.18 to 7.49. In 9 of the 12 cases, the ratio is 4 or more, making the odds extremely high that the 2 inbred strains reported are significantly less variable in height of plant than is the parental variety.

In table 2 are presented the odds according to Fisher that the differences between the variability of the parental check rows and each respective inbred progeny from the same variety are significant. In all of the 12 comparisons the odds are 99:1, or only slightly less. In 6 of the 12 cases Fisher's 1 percent point is distinctly surpassed. There is little doubt, therefore, that these inbred progenies are distinctly less variable than the parental variety as represented by a check row of 35 to 38 plants.

TABLE 2.—*Coefficients of variability (C.V.) and the odds by Fisher's z test that there is a significant difference in the variability of plant height between the parental variety (check) and inbred progenies from the variety designated*

Variety progeny	Number of plants	C.V.	Approximate odds [1]	Variety progeny	Number of plants	C.V.	Approximate odds [1]
Utah Common:				Hardigan:			
Check	35	21.8		Check	35	22.0	
No. 127	8	9.5	−99:1	No. 125	7	9.1	−99:1
No. 61	18	12.9	+99:1	No. 17	36	12.3	+99:1
Grimm:				Dakota Common:			
Check	37	22.9		Check	35	21.9	
No. 42	16	12.2	+99:1	No. 22	11	11.1	−99:1
No. 52	19	14.9	−99:1	No. 67	40	13.8	+99:1
Saskatchewan No. 666:				Ontario Variegated:			
Check	38	26.9		Check	35	25.0	
No. 4	35	9.3	+99:1	No. 136	8	7.9	+99:1
No. 51	12	13.4	+99:1	No. 25	36	14.3	+99:1

[1] The minus sign before 99:1 in this and in subsequent tables indicates that the odds are much nearer 99:1 than they are to 19:1; a plus sign before 19:1 indicates that the odds are greater than 19:1 but nearer to that than to 99:1.

WIDTH OF PLANT

Data regarding plant width are similarly summarized by classes in table 3. Parental varieties all have coefficients of variability which put them in the 22.5- to 32.5-percent classes, whereas about one-third of the progenies from each of the six varieties have coefficients of variability as low as 12.5 percent; in Hardigan there was one progeny as low as 7.5 percent. On the whole, there seem to be more progenies in Saskatchewan No. 666 that show low variability.

Comparisons were again made of coefficients of variability of the different parental varieties with those of the respective inbred progenies and the difference was divided by the probable error of the difference. Of the 12 ratios of Diff./P.E., there are 3 below 3 and 9 above 3, with 7 that are 4 or more. An examination of these ratios indicates high odds that the inbred progenies from Utah Common, Grimm, Saskatchewan No. 666, and Hardigan are significantly less variable for plant width than is the parental stock. In Ontario Variegated, the ratios of Diff./P.E. are from just under 3 to just over 4. In Dakota Common, however, one ratio is well below 2 and the other is 2.99.

TABLE 3.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to plant-width classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*

C.V. class (percent)	Number of progenies in indicated plant-width class in inches									
	14	16	18	20	22	24	26	28	30	32
UTAH COMMON										
7.5										
12.5				1						
17.5			2	3	1	1				
22.5	1		2	2	2	1				
27.5		2	1	1x	1					
32.5	2	1	1	1						
37.5		1		1		1				
42.5				1						
GRIMM										
12.5				1	1					
17.5				1	2					
22.5		1	3							
27.5		1	1x	1						
32.5				1						
37.5										
42.5										
47.5		1								
SASKATCHEWAN NO. 666										
12.5			1	2	1					
17.5		1		1	1	1	1			
22.5		2	1	2	2	3				
27.5	1			4	1	1				
32.5		1	x		1					
37.5		2								
42.5										
HARDIGAN										
7.5				1						
12.5				1	1					
17.5					2					
22.5			1	x						
27.5			1	1						
32.5		1	1				1			
37.5										
42.5										
47.5										
52.5				1						

TABLE 3.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to plant-width classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*—Continued

C.V. class (percent)	Number of progenies in indicated plant-width class in inches									
	14	16	18	20	22	24	26	28	30	32
DAKOTA COMMON										
17.5					1		1			
22.5			1	1		1	1			
27.5			1	1	*x*					
32.5					1					
ONTARIO VARIEGATED										
12.5						1				
17.5						1				
22.5			1		2					
27.5				*x*	1					

Table 4 shows the probability by Fisher's *z* test to be 99:1 or more that the inbred progenies are less variable than the parental stock of Grimm, Saskatchewan No. 666, and Hardigan. The least variable progeny of Ontario Variegated surpasses the 1 percent point, whereas the other progeny of Ontario Variegated and the least variable progeny of Utah Common and Dakota Common show odds between 19:1 and 99:1. The second least variable progeny of Utah Common and of Dakota Common gave odds of less than 19:1. Three of the six varieties show high probability that the inbred progenies are less variable in plant width than the parental variety.

TABLE 4.—*Coefficients of variability (C.V.) and the odds by Fisher's z test that there is a significant difference in the variability of plant width between the parental variety (check) and inbred progenies from the variety designated*

Variety progeny	Number of plants	C.V.	Approximate odds [1]
Utah Common:			
Check	35	26.1	Between 19:1 and 99:1.
No. 103	11	13.8	
No. 60	9	15.5	−19:1.
Grimm:			
Check	37	26.9	
No. 42	16	12.9	+99:1.
No. 53	17	15.4	−99:1.
Saskatchewan No. 666:			
Check	38	31.4	
No. 4	35	12.6	+99:1.
No. 5	38	15.6	+99:1.
Hardigan:			
Check	35	24.2	
No. 16	29	7.5	+99:1.
No. 15	40	13.8	+99:1.
Dakota Common:			
Check	35	25.8	
No. 22	40	18.3	Between 19:1 and 99:1.
No. 47	21	19.8	−19:1.
Ontario Variegated:			
Check	35	25.3	
No. 69	16	12.3	+99:1.
No. 68	23	17.2	+19:1.

[1] See footnote 1, table 2.

ERECTNESS OF PLANT

Data for plant erectness, as shown in table 5, likewise indicate decreased variability as a result of inbreeding. The parental material in Dakota Common, however, was no more variable than the least variable progeny. In Hardigan, only 3 of the 15 progenies were less variable than the parent plants, and these not significantly so. In the other varieties, coefficients of variability of the parent rows ranged from 15 to 24 percent, whereas a considerable number of the progenies had a variability of 12 percent or lower. The tendency toward uniformity as a result of inbreeding is less marked for plant erectness than for plant height or plant width, except in Saskatchewan No. 666, which seems to have become as relatively uniform in erectness as in height or width. In view of the previous close breeding in Saskatchewan No. 666, this is just what might be expected if erectness depends on a greater number of factors than does height or width.

As measured by Diff./P.E., the inbred progenies of Utah Common, Saskatchewan No. 666, and Ontario Variegated seem to be distinctly less variable than the respective check rows, since the ratios of the difference to the errors are all considerably above 4, and two are above 7, one being 8.26. The ratio of Diff./P.E. for the progenies from Grimm are about 3 and for Hardigan from just below 3 (2.96) down almost to 1. For Dakota Common, the error is greater than the difference.

TABLE 5.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to plant-erectness classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*

C.V. class (percent)	Number of progenies in indicated plant-erectness class in degrees											
	28	32	36	40	44	48	52	56	60	64	68	72
UTAH COMMON												
6					1	1	1					
9						2	1	2				
12					1		1	1		1		
15				1	2	4						
18				2	2	3x						
21			1			1	1					
24				1								
GRIMM												
9					2							
12							1					
15					1	1x						
18					1			1				
21								1	1			
24					1		1					
27												
30				2								
33		1										

TABLE 5.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to plant-erectness classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*—Continued

C.V. class (percent)	Number of progenies in indicated plant-erectness class in degrees											
	28	32	36	40	44	48	52	56	60	64	68	72
SASKATCHEWAN NO. 666												
9					1	1			1			
12				1	1	2						
15			1	1	1	2	1	1				
18			1	2	1	1		1				
21				1	2		1					
24		1			1*x*	1						
27				1								
30			1									
33												
36		1										
39												
HARDIGAN												
9					1							
12						1		1				
15					1	1*x*	1	2	1			
18							1					
21					1							
24												
27												
30												
33												
36				1								
DAKOTA COMMON												
9						1	*x*		1			1
12						1	2					
15						1						
18					1	1						
ONTARIO VARIEGATED												
6							1					
9						1	1					
12						1			1			
15												
18												
21				1		*x*						

As measured in table 6 by the *z* test, Utah Common, Saskatchewan No. 666, and Ontario Variegated all show odds greater than 99:1 that the inbred progenies are less variable. In Grimm the odds are more than 19:1 and just less than 19:1 for the two progenies. In Hardigan the odds for both progenies are less than 19:1. In Dakota Common the parental stock was not highly variable for plant erectness, being no more variable than the least variable inbred progeny.

Plant erectness in 3 of 6 varieties yielded less rapidly to inbreeding than did plant height or plant width. Various types of plants are shown in figures 1 and 2.

TABLE 6.—*Coefficients of variability (C.V.) and the odds by Fisher's z test that there is a significant difference in the variability of plant erectness between the parental variety (check) and inbred progenies from the variety designated*

Variety progeny	Number of plants	C.V.	Approximate odds [1]
Utah Common:			
Check	35	17.0	
No. 76	17	5.4	+99:1.
No. 1	38	9.9	+99:1.
Grimm:			
Check	37	15.0	Between 19:1 and 99:1.
No. 33	17	9.3	
No. 5	16	10.4	−19:1.
Saskatchewan No. 666:			
Check	38	25.3	
No. 5	38	9.1	−99:1.
No. 33	27	11.8	+99:1.
Hardigan:			
Check	35	14.2	
No. 125	7	8.5	−19:1.
No. 16	29	12.2	−19:1.
Dakota Common:			
Check	35	7.6	
No. 22	40	7.9	Very low.
Ontario Variegated:			
Check	35	20.0	
No. 136	8	4.7	+99:1.
No. 68	23	7.8	+99:1.

[1] See footnote 1, table 2.

DIAMETER OF STEM

Stem diameter responded readily to inbreeding by showing striking decreases in variability (table 7). Whereas parent varieties showed a variability of from 16 to 20 percent, about a fifth to a fourth of the progenies from each variety had coefficients of variability of 12 percent or less. There is strong evidence in the Diff./P.E. ratios that inbred progenies are distinctly less variable than parental stock. The Diff./P.E. varies from 3.77 to 6.05 in 10 of the 12 cases, 8 of them being more than 4. Only the one comparison for Dakota Common is less than 3, this being 2.79.

TABLE 7.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to stem-diameter classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*

C.V. class (percent)	Number of progenies in indicated stem-diameter class in millimeters											
	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9	5.1	5.3	5.5
UTAH COMMON												
6									1			
8								1	1			
10								1	1			
12					1	1	1	1	1			
14					1	2		4		2	1	
16				1	1		1				1	
18							1	1x		1		
20	1							1				
22							1					
24												

TABLE 7.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to stem-diameter classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*—Continued

C.V. class (percent)	Number of progenies in indicated stem-diameter class in millimeters											
	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9	5.1	5.3	5.5
GRIMM												
8							1					
10							1					
12				1		2						
14				2								
16				1				1				1
18					2	*x*		1				
20												
22							1					
SASKATCHEWAN NO. 666												
6			1									
8								1			1	
10												
12				2	1				1			
14				1		1	3				1	
16			1		2			1				
18					2	3	1					
20				1	*x*	1	1		1	1		
22	1											
24							1					
26												
HARDIGAN												
8												
10							1					
12						1				1		
14												
16							1	2		1	1	
18								1				
20						*x*						
22				1			1					
40									1			
DAKOTA COMMON												
12							1	2				
14							1				1	
16								1*z*		1		
18									1			
20								1				
ONTARIO VARIEGATED												
10							1					
12							1					
14								1		1		1
16							1					
18												
20					*x*							

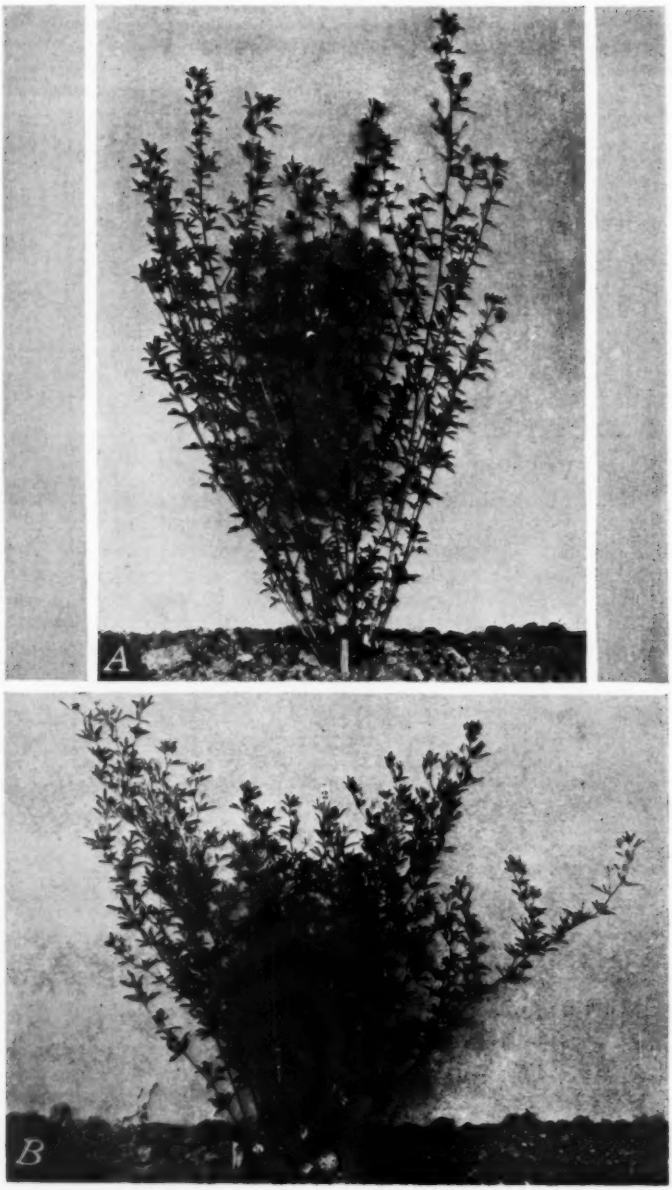

FIGURE 1.—*A*, An erect, moderately leafy plant; one progeny of Dakota Common was almost pure for this type of plant. *B*, A moderately erect, rather leafy plant; several progenies were nearly pure for this type of plant.

Table 8 shows that the odds by the z test all exceed 19:1 and 6 of the 12 exceed 99:1. The odds are smallest for Dakota Common, both progenies slightly exceeding the 5-percent point. Such con-

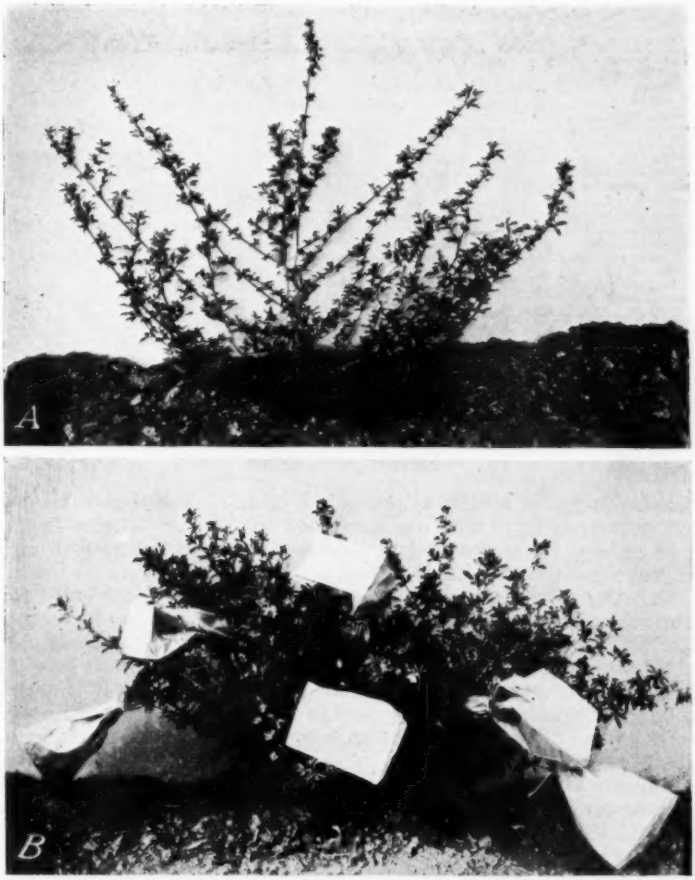

FIGURE 2.—*A*, A rather spreading plant low in percentage of leaves and high in percentage of stalks; one progeny was almost pure for this plant type. *B*, A low, spreading plant with fine stems and a high percentage of leaves; all varieties gave one or more progenies nearly pure for this plant type. The manner of bagging to control pollination is shown.

sistently high odds leave little doubt that in stem diameter inbreeding for one generation has decreased the variability of the more nearly uniform progenies.

TABLE 8.—*Coefficients of variability (C.V.) and the odds by Fisher's z test that there is a significant difference in the variability of stem diameter between the parental variety (check) and inbred progencies from the variety designated*

Variety progeny	Number of plants	C.V.	Approximate odds [1]
Utah Common:			
Check	35	18.0	
No. 129	7	6.5	+99:1.
No. 103	11	8.1	+99:1.
Grimm:			
Check	37	18.8	
No. 112	7	8.0	Between 19:1 and 99:1.
No. 42	16	10.5	99:1.
Saskatchewan No. 666:			
Check	38	19.2	
No. 153	7	8.6	+19:1.
No. 82	17	11.5	Between 19:1 and 99:1.
Hardigan:			
Check	35	19.3	
No. 16	29	10.4	+99:1.
No. 18	40	12.0	+99:1.
Dakota Common:			
Check	35	16.2	
No. 21	40	11.7	+19:1.
No. 22	40	11.3	+19:1.
Ontario Variegated:			
Check	35	20.8	
No. 136	8	9.8	Between 19 and 99:1.
No. 24	39	12.4	+99:1.

[1] See footnote 1, table 2.

LENGTH OF LEAFLET

Data for leaflet length as presented in table 9 also show segregation in variability. The variability of the parents ranges from 14 to 18 percent for leaflet length, whereas one-third to one-fifth of the progenies have a variability as low as 10 percent or less.

The strong tendency for the least variable inbred progenies to be more uniform in leaflet length than the parental stock is shown in the ratio Diff./P.E., which ranges from 3.60 to 6.45 in 10 of the 12 cases, being more than 4 in 7 cases. The second most uniform progenies show a ratio of less than 2 for Dakota Common. Table 10 shows the probability according to the *z* test to be 99:1, or nearly so, in 10 of the 12 cases. The second most uniform progeny from Utah Common with 8 plants and the second most uniform progeny from Dakota Common with 40 plants both showed odds of less than 19:1. Only one progeny in Dakota Common had a significantly lower variability than did the check row. The variability of the check row in this variety was only about two-thirds as great as it was in most of the other parental varieties, i.e., the parental material itself had a low variability for this character.

TABLE 9.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to leaflet-length classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*

C.V. class (percent)	Number of progenies in indicated leaflet-length class in millimeters							
	22	24	26	28	30	32	34	36
UTAH COMMON								
6				1	3			
8			1	1	1	1		
10		1			1	1		
12			1	1	1	2		
14		1	1	4	2*x*			
16				3		2		
18					1			
GRIMM								
8					1	1		
10				1	1			
12	1		2	1				
14		1	2					
16			1					
18			1*x*					
20			1					
SASKATCHEWAN NO. 666								
6					2			
8				1				
10		1		2				
12		1	1	1	1	1		
14	1		1	1				
16		4	3					
18	1	1*x*	2	1	1			
20			1					
22								
24								
26								
28				1				
30								
32								
34								1
HARDIGAN								
10					3			
12			1		3	2		
14			1	*x*	1	1		
16								
18								
20								
22								
24								
26								
DAKOTA COMMON								
4								
8								
10				1	1			
12			1	2*x*				
14				1				
16				1		1		
18				1				

TABLE 9.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to leaflet-length classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*—Continued

C.V. class (percent)	Number of progenies in indicated leaflet-length class in millimeters							
	22	24	26	28	30	32	34	36
ONTARIO VARIEGATED								
6				1				
8								
10				1	1			
12				1			1	
14								
16			1	x				
18				x				

TABLE 10.—*Coefficients of variability (C.V.) and the odds by Fisher's z test that there is a significant difference in the variability of leaflet length between the parental variety (check) and inbred progenies from the variety designated*

Variety progeny	Number of plants	C.V.	Approximate odds [1]	Variety progeny	Number of plants	C.V.	Approximate odds [1]
Utah Common:				Hardigan:			
Check	35	13.2		Check	35	14.9	
No. 76	17	6.6	+99:1	No. 17	36	9.5	+99:1
No. 127	8	7.3	−19:1	No. 16	29	10.3	−99:1
Grimm:				Dakota Common:			
Check	37	17.2		Check	35	12.1	
No. 11	37	8.5	+99:1	No. 133	7	4.8	−99:1
No. 42	16	10.3	−99:1	No. 23	40	10.1	−19:1
Saskatchewan No. 666:				Ontario Variegated:			
Check	38	17.6		Check	35	17.8	
No. 153	7	6.1	+99:1	No. 16	16	9.2	+99:1
No. 6	37	10.5	+99:1	No. 25	36	10.2	+99:1

[1] See footnote 1, table 2.

WIDTH OF LEAFLET

In leaflet width (table 11) the varieties range from 21 to 27 percent in variability as compared to a variability of 15 percent or less for about a fifth of the progenies.

The tendency to uniformity seems to be even stronger in leaflet width than in the characters already studied; all ratios of Diff./P.E. except one are greater than 3, and one is 8.23. In Saskatchewan No. 666, the third least variable class still shows a ratio of 3. The second least variable progeny of Ontario Variegated shows a ratio of 2.68, the only instance in which there is a ratio less than 3. Figure 3 shows variations in leaflet length and width.

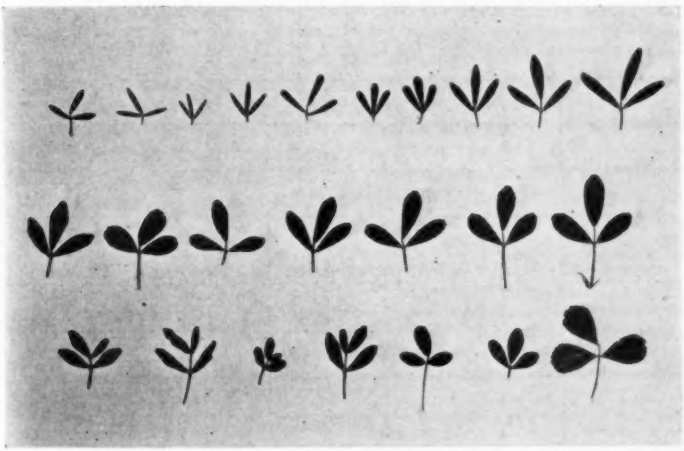

FIGURE 3.—Variations in leaflet length and width; nearly pure-breeding progenies occurred similar to the 2 on the extreme right in all 3 rows and the 1 on extreme left in the middle row.

TABLE 11.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to leaflet-width classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*

C.V. class (percent)	Number of progenies in indicated leaflet-width classes in millimeters									
	7	8	9	10	11	12	13	14	15	16
UTAH COMMON										
6						1				
9					1			1		
12					1			3		1
15						1	1	2		
18				2	1		3	3		
21				1	2	2	1x			
24								1		
27									1	
30									1	
GRIMM										
9				1						
12					2			1		
15					1					
18					3		1			
21				1	1					
24					2x					
27					1					
SASKATCHEWAN NO. 666										
9										
12				1	1	1	2			
15			1	1	2	1				
18			1	2	1	5				1
21	1			3	2	3				
24				x			1			

TABLE 11.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to leaflet-width classes and coefficient of variability (C.V.) classes, grown at Fort Duchesne, Utah, 1930*—Continued

C. V. class (percent)	Number of progenies in indicated leaflet-width classes in millimeters									
	7	8	9	10	11	12	13	14	15	16
HARDIGAN										
15						1				
18						2		1		
21						2	1	1		
24						1			1	
27				1		*x*				
30						1				
DAKOTA COMMON										
12						1				
15						2	1	1	1	
18										
21							*x*		1	
24										
27										
30							1		1	
ONTARIO VARIEGATED										
12						1				
15					1	1	1	1		
18								1		
21							*x*			

In 10 of the 13 comparisons, presented in table 12 odds, according to the *z* test, are 99:1, or practically so. One progeny each in Dakota Common and in Ontario Variegated shows odds of about 19:1. In Saskatchewan No. 666, a progeny from the third least variable class still shows odds of more than 19:1 that it is less variable than the parent stock.

It is clear that for leaflet width one generation of inbreeding has reduced the variability of a considerable proportion of the progenies.

TABLE 12.—*Coefficients of variability (C.V.) and the odds by Fisher's z test that there is a significant difference in the variability of leaflet width between the parental variety (check) and inbred progenies from the variety designated*

Variety progeny	Number of plants	C.V.	Approximate odds[1]	Variety progeny	Number of plants	C.V.	Approximate odds[1]
Utah Common:				Hardigan:			
Check	35	21.8		Check	35	25.7	
No 142	9	5.2	+99:1	No 15	40	15.9	+99:1
No. 62	12	10.3	+99:1	No. 14	40	16.5	+99:1
Grimm:				Dakota Common:			
Check	37	23.6		Check	35	21.4	
No. 112	7	9.3	−99:1	No. 67	11	12.3	+19:1
No. 11	12	10.3	+99:1	No. 22	40	14.0	99:1
Saskatchewan No. 666:				Ontario Variegated:			
Check	38	24.8		Check	35	21.3	
No 5	38	12.9	+99:1	No. 136	8	11.7	19:1
No. 34	26	13.8	+99:1	No. 24	39	15.5	−99:1
No. 8	12	14.8	+19:1				

[1] See footnote 1, table 2.

COLOR OF BLOSSOM

Inbreeding has likewise produced progenies in which the variability in blossom color is so greatly reduced as to be practically pure breeding. Some of these low-variability strains are essentially uniform for light violet-colored blossoms, others for an intermediate violet color, and still others for a dark violet. It will be noted (see below) that Utah Common produced progenies true-breeding for 5 different grades of violet color, and Grimm and Saskatchewan No. 666 for 4 grades each. In order to give the colors numerical values, a study was made of Ridgway's plate XXV, on which are found the color standards that matched the violet alfalfa blossoms. With the help of a competent artist, colors were assigned the values shown in table 13.

TABLE 13.—*Color values used in classifying alfalfa blossoms, and the names of the colors*

[The designations of column headings and the letters in the first column are Ridgway's. The numerals in the other columns are arbitrary values indicated by consecutive numbers, used to permit calculation by standard deviation and coefficient of variability]

Grade of color	61′ VR–V	63′ R–V	65′ RR–V
White	0 (white)	0 (white)	0 (white).
f	2 (pale lavender violet)	3 (pale mauve)	4 (mauvette).
d	5 (light lavender-violet)	6 (light mauve)	7 (lilac).
b	8 (lavender violet)	9 (mauve)	10 (Chinese violet).
——	11 (pleroma violet)	12 (manganese violet)	13 (Mathews' purple).
i	14 (haemotoxylin violet)	15 (litho purple)	16 (petunia violet).
k	17 (anthracene violet)	18 (madder violet)	19 (nigrosin violet).
m	20 (dark anthracene violet)	21 (dark madder violet)	22 (dark nigrosin violet).

A few of the blossoms from variegated strains were matched by the standards on plate XXIV. The violet colors in both common and variegated strains ranged from the color value 6 (63′ *R–V*, *d*, that is, light mauve) to a value of 22 (65′ *RR–V*, *m*, that is, dark nigrosin violet). Extremely light variegated colors were assigned values between 0 and 6. Only a few blossoms even in the variegated strains were outside the range 6 to 22. The blue-violet, blue-green, yellow-green, and smoky colors were assigned values from 24 to 40; plain yellow was assigned the value 42. Within the limits mentioned, the larger the number of the color value the darker the color, by rather uniform steps. The numbers assigned to the variegated colors (24 to 40) are more arbitrary, and although they occur only rarely they express wide variation when they do occur.

For the Utah Common parental variety, table 14 shows the mean color value to be 10 and the standard deviation to be 3. As compared with this, one progeny had a standard deviation (used because the means were assumed values) of less than 0.5 of 1 percent. Almost, but not quite, as uniform were 16 other progenies which had standard deviations between 0.5 and 1.49. These practically pure-breeding progenies showed distinct differences in darkness of violet color. One was so light as to be classed as 6 (63′ *R–V*, *d*, light mauve), 4 were classed as 10 (65′ *RR–V*, *b*, Chinese violet), 7 as 12 (63′ *R–V*, manganese violet), 3 as 14 (61′ *VR–V*, *i*, haematoxylin violet), and 1 as 16 (65′ *RR–V*, *i*, petunia violet).

TABLE 14.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to blossom-color classes and standard-deviation (S.D.) classes, grown at Fort Duchesne, Utah, 1930*

S.D. class (percent)	Number of progenies in blossom-color classes—[1]											
	6	8	10	12	14	16	18	20	22	24	26	28
UTAH COMMON												
0				1								
1	1		4	7	3	1						
2	1			3	2							
3		1	1*x*	2		1						
4				1								
5												
6					1							
GRIMM												
1			2	2	1							
2				1		1						
3												
4					1		1					
5												
6												
7			1									
8							1					
9								1				
10						*x*	1					
11												
12							1					
SASKATCHEWAN NO. 666												
0												
1		3	1	4	1							
2	1	1		3								
3			2		1							
4												
5					2	1						
6	1		1*x*					1				
7				1								
8				1								
9							1				1	
10								1	1			
11					1							
12												
HARDIGAN												
0			1				1					
1			3	2								
2					1							
3												
4					1							
5					1*x*							
6												
7						1						1
DAKOTA COMMON												
0					1							
1			1	1	2							
2			1	1*x*	1		1					

[1] See text and table 13.

TABLE 14.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to blossom-color classes and standard-deviation (S.D.) classes, grown at Fort Duchesne, Utah, 1930*—Continued

S.D. class (percent)	Number of progenies in blossom-color classes—											
	6	8	10	12	14	16	18	20	22	24	26	28
ONTARIO VARIEGATED												
0												
1				2								
2				1								
3					1							
4												
5												
6				1	*x*							
7												
8									1			

In Grimm progenies the segregation was especially noticeable. Grimm had a mean color value of 16 with a standard deviation of 10, indicating high variability. This is to be expected since Grimm is a variegated variety. It is highly significant that 7 of the 14 Grimm progenies became essentially true-breeding for blossom color in one generation of inbreeding. The mean color values of these low-variability segregates were 10, 12, 14, and 16 and the standard deviations were either 1 or 2. Two other progenies had standard deviations of 4. Five progenies approached the parental row in degree of variability.

What has been said for Grimm might also be said for Saskatchewan No. 666, except it should be added that the variability of the parent variety is expressed by a standard deviation of 6 and by a mean color value of 10. Nine, nearly if not fully, pure-breeding progenies (S.D.=1) were obtained for colors whose value is expressed by every class from 8 to 14. There was, therefore, a distinct segregation toward several colors that were less variable than the parent stock. The range for nonuniform darker colors extended considerably beyond those obtained from Grimm but with the same degree of variability (S.D. ranged from 8 to 12).

So far as can be learned from comparatively small numbers of progenies, Hardigan and Ontario Variegated behaved in a manner similar to Saskatchewan No. 666. The mean color values of the parent rows were 14 and the standard deviations 5 and 6, respectively. Both gave nearly true-breeding progenies, about two-thirds of each group being less than half as variable as the parent stock. One 40-plant progeny of Hardigan had a standard deviation of 0, being completely uniform to the degree of fineness that could be measured by the color charts.

Dakota Common parent stock had a color value of 12 and a standard deviation of 2. Even with this low variability and in a total of only nine progenies, there was a real tendency for some progenies to be less variable than the parent stock. One progeny had a standard deviation of less than 0.5 and four of less than 1.

The ratios of Diff/P.E. are so distinctly outstanding as to leave no doubt of segregation for greater uniformity. In 10 of the 12 cases, the ratios vary from 7.68 to 12.41. The other two are 6.14 and 4.70.

TABLE 15.—*Standard deviations (S.D.) and the odds by Fisher's z test that there is a significant difference in the variability of blossom color between the parental variety (check) and inbred progenies from the variety designated*

Variety progeny	Number of plants	Standard deviation	Approximate odds [1]	Variety progeny	Number of plants	Standard deviation	Approximate odds [1]
Utah Common:				Hardigan:			
Check	35	2.65		Check	35	5.87	
No. 129	7	0	+99:1	No. 18	40	0	+99:1
No. 20	40	1.22	+99:1	No. 15	40	1.48	+99:1
Grimm:				Dakota Common:			
Check	37	10.80		Check	35	2.37	
No. 11	37	1.10	+99:1	No. 65	76	0.75	+99:1
No. 53	17	2.10	+99:1	No. 23	36	1.34	+99:1
Saskatchewan No. 666:				Ontario Variegated:			
Check	38	6.66		Check	35	6.33	
No. 6	37	1.38	+99:1	No. 68	23	1.10	+99:1
No. 81	19	2.17	+99:1	No. 136	8	1.34	+99:1

[1] See footnote 1, table 2.

As shown in table 15, the odds are all more than 99:1, and several of them are probably thousands to one, that blossom color in many progenies has become almost uniform as a result of one generation of controlled inbreeding. Though statistically calculated for only 12 progenies the odds are approximately as high for 36 others, that is, for 48 in all, or nearly half.

COLOR OF FOLIAGE

Foliage color was also found to segregate and to become notably less variable as a result of one generation of inbreeding. The mean color values (checked as in blossom color by Ridgway's color plate XVII) were all 17, 18, or 19, with the following standard deviations: 3 for Utah Common, Saskatchewan No. 666, and Hardigan; 4 for Grimm; and 1 for Dakota Common and Ontario Variegated. All varieties produced several progenies which had a low variability as compared with the parent stock. Several 30- to 40-plant progenies had standard deviations of 0, being completely uniform by chart variations for colors 19, 21, and 22, which are bice green, elm green, and forest green. These differences are clearly distinguishable to the eye when the plants are grown in rows close together. None of the varieties yielded progenies distinctly more variable than the parent stock, and all of them seemed to segregate for 3 or 4 grades of leaf color, usually toward the darker shades, though one progeny of Utah Common had leaves which were distinctly lighter in color than the parental plants (table 16).

The evidence for segregation toward greater uniformity of foliage color is even greater than for blossom color. The ratios of Diff./P.E. are all above 7.44, and 11 of them are above 10. In 7 of the 14 cases the progenies were found to be entirely true-breeding so far as could be determined by the color charts, as well as 14 others not so studied. In addition, 27 other progenies had extremely low variability. Table 17 shows the probabilities by the *z* test to be all over 99:1 and at least half of them many thousands to one.

TABLE 16.—*Inbred progenies of different alfalfa varieties and check row of parent plants (x) arranged according to leaf-color classes and standard-deviation (S.D.) classes, grown at Fort Duchesne, Utah, 1930*

S.D. class	Number of progenies in leaf-color class [1]—						
	16	17	18	19	20	21	22
UTAH COMMON							
0				3		4	1
0.50				1	4	7	
1	1		1	4	3	1	
2							
3			*x*				
4							
GRIMM							
0						1	1
0.50				1	3	1	
1			1	3	3		
2							
3							
4				*x*			
SASKATCHEWAN NO. 666							
0				2		4	
0.50				4	3	8	
1				3	6		
2							
3				*x*			
HARDIGAN							
0				2			
0.50				2	1	2	
1			1	1	1		
2							
3				*x*			
4							
5					2		
DAKOTA COMMON							
0				1			1
0.50					2	2	
1				*x*	3		
ONTARIO VARIEGATED							
0				1			
0.50					1	1	
1				*x*		1	
2						1	
3							

[1] See text.

COLORATIONS AND PECULIARITIES

About one-fifth of the progenies had a strong tendency to develop distinct reddish or reddish-brown colorations on the stems. About one-fifth produced no such color markings at all; the remaining three-fifths produced them irregularly.

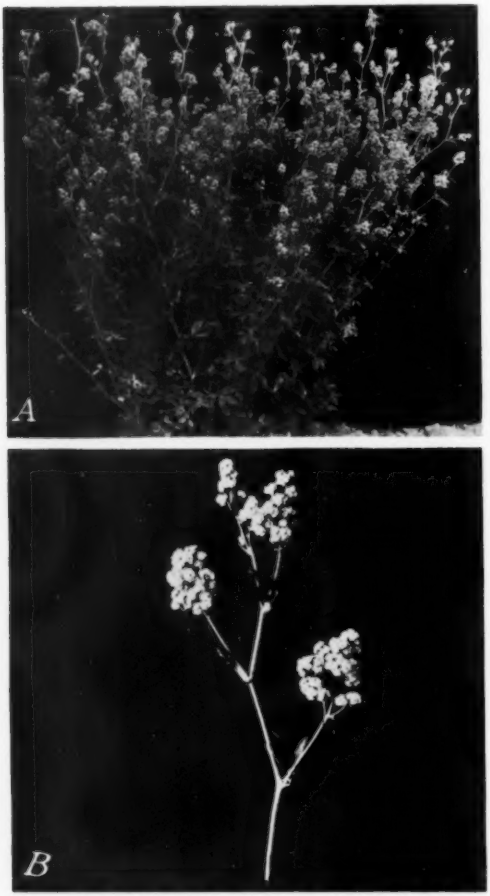

FIGURE 4.—*A*, Alfalfa plant with blossoms entirely replaced by vegetative proliferation described in text. *B*, Close view of one branch from plant shown in *A*.

One 30-plant progeny produced on almost exactly half the plants a freak mealy vegetative proliferation of the flower which completely replaced the blossoms and resembled in appearance the flower buds of seed onions before they open (fig. 4). This proliferation is seen at rare intervals in the field but is more abundant by thousands of times, perhaps by millions of times, in this inbred progeny than under ordinary field conditions.

About a dozen progenies contained from 1 to 10 plants on which the ends of the uppermost internodes were longer and slenderer than ordinary. Frequently, these "viny" top internodes gave the plants an appearance somewhat suggestive of vetch.

There was a wide segregation for tall and short, for leafy and stalky, and for fine- and coarse-stemmed plants, which indicates a wide opportunity for forage selections. There was also a strong suggestion of segregation for seed production.

TABLE 17.—*Standard deviations (S.D.) and the odds by Fisher's z test that there is a significant difference in the variability of leaf color between the parental variety (check) and inbred progenies from the variety designated*

Variety progeny	Number of plants	Standard deviation	Approximate odds [1]	Variety progeny	Number of plants	Standard deviation	Approximate odds [1]
Utah Common:				Hardigan:			
Check	35	3.23		Check	35	3.28	
No. 19	27	0	+99:1	No. 58	12	0	+99:1
No. 29	31	0	+99:1	No. 14	40	.28	+99:1
No. 28	21	.77	+99:1	Dakota Common:			
Grimm:				Check	35	1.58	
Check	37	4.07		No. 21	40	0	+99:1
No. 16	16	.24	+99:1	No. 23	36	.50	+99:1
No. 13	49	.77	+99:1	Ontario Variegated:			
Saskatchewan No. 666:				Check	35	1.00	
Check	38	3.76		No. 136	8	0	+99:1
No. 5	38	0	+99:1	No. 25	36	.36	+99:1
No. 6	37	0	+99:1				
No. 34	27	.57	+99:1				

[1] See footnote 1, table 2.

SUMMARY AND CONCLUSIONS

To determine the effect of inbreeding on variability in alfalfa, eight characters were studied in each of six varieties. A summary of all ratios of Diff./P.E. for coefficients of variability or standard deviations when the inbred progenies are compared with the parental stock shows that of 96 progeny characters studied only 10 had ratios of less than 3, and of these, 6 were in the progenies of one variety, Dakota Common. Of the 96 comparisons, 46 had ratios of Diff./P.E. of 5 or more. An equally large or larger number of progenies not studied statistically had variabilities approximately as low as those studied. The particular stock of Dakota Common was observed to be rather uniform, and in the data this was shown in the coefficient of variability in 4 characters. The inbred progenies had as low variability for the characters in question as did the progenies in the other varieties. In the 4 other characters Dakota Common was about as variable as the other varieties and showed equally large ratios of Diff./P.E.

Ninety-six comparisons were made by Fisher's z test, 75 of which showed odds of approximately 99:1 or more that a considerable group of the least variable progenies were less variable than the parent stock from which they were derived. A large group not studied statistically had very low variability. In 12 of the comparisons the odds were between 19:1 and 99:1; in 9 the odds were below 19:1. Of these 9, 6 were for progenies from the one variety Dakota Common, the varietal stock of which was found to be low in variability for 4 plant characters and normally variable in 4 others.

It is worthy of note that a high degree of correspondence was found between the results obtained by the probable-error method and the z test. In fact so close was the correspondence both for coefficients of variability and for standard deviations that either method almost fully duplicates the other.

The significantly lower variability in progenies with respect to plant height, plant width, stem diameter, leaflet length and width, blossom color, and foliage color as a result of one generation of inbreeding leaves little doubt that alfalfa is much less heterozygous than it has been commonly thought to be. Blossom color segregated

widely, the progenies grading from extremely light violet to extremely dark violet. Many of them were practically pure-breeding. Even the variegated varieties yielded nearly uniform blossom color in a high percentage of the progenies.

Saskatchewan No. 666, which had been close-bred for some years before this experiment began, showed more uniformity in most plant characters, indicating that further generations were required to complete the segregation. This was especially clear with plant erectness, in that those strains not previously close-bred produced fewer progenies indicating low variability, and even these seemed to be less clearly removed from the variability of the parent stock than were the other plant characters. Erectness also appears to be more complex genetically than at least part of the other characters, such as leaflet length and width, blossom color, and foliage color.

The wide variation in forage characters suggests an unusually promising economic opportunity in alfalfa breeding. This is also true for seed production. Incidentally these data reported here put a rather hopeful aspect on crossbreeding followed by inbreeding as a means of uniting resistance to bacterial wilt with desirable agronomic qualities.

DISTRIBUTION OF SOIL MOISTURE UNDER ISOLATED FOREST TREES[1]

By HERBERT A. LUNT

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INTRODUCTION

That soil moisture is usually the chief limiting factor in forest-tree growth is generally recognized. This may be as true in a local area affecting a few individual trees as it is in a larger climatic region. Irregularities in the topography, the presence of decaying stumps, depressions left by the up-rooting of wind-blown trees, and the severe and unequal competition of the surrounding trees, all contribute to the lack of uniformity generally characteristic of undisturbed stands of timber. These facts make the study of soil-moisture relations in the forest extremely difficult. For this reason better results may be expected from studies made in carefully selected forests, which are uniform as to soil, topography, and forest stand, such as might be obtained in a plantation. In some instances individual isolated trees can be used to advantage, and this was done in the present study.

The work of Conrad and Veihmeyer in California [2] on root development and soil moisture, carried out with sorghum plants, led the author to attempt a somewhat similar study with forest trees. It is recognized that the California type of climate, characterized by little or no rainfall during the growing season, is the ideal condition for this sort of work. Nevertheless, it was felt that such a study would be of value in humid New England in spite of its frequent summer showers. The samplings were made only during the hottest and driest part of the summer, as near as possible to the end of a prolonged dry period.

PROCEDURE

The general plan was to dig a trench from the base of a tree out into the open and to sample the soil at several depths and at definite intervals from the tree. The sampling was done with a brass-sampling tube three-fourths inch in diameter, and each sample was a composite from borings made on the two opposite sides of the trench. Immediately after a sample was taken it was placed in a small tin box with a tight-fitting cover, carried to the laboratory, weighed, dried, and reweighed. Later, moisture-equivalent determinations were made on most of the samples. The work consisted of 4 separate experiments, 2 under pine and 2 under oak.

PRESENTATION OF DATA

EXPERIMENT 1

The first experiment was made under red pine, *Pinus resinosa*, in block 16, Rainbow plantation, planted in 1917. The samples were taken July 22, 1930. The tree selected, which was approximately

[1] Received for publication June 1, 1934; issued November 1934.

[2] CONRAD, J. P., and VEIHMEYER, F. J. ROOT DEVELOPMENT AND SOIL MOISTURE. Hilgardia 4:113-134, illus. 1929.

16½ feet in height at the time of sampling, with an 8-foot spread, stood at the end of a row on the side of a block of red pine and suppressed white pine. Samples were taken (1) at 1, 2, 3, 4, 5, 6, 7, and 8 feet out from the tree, and at depths of 2, 7, 12, 18, 24, 30, and 36 inches; (2) on the opposite side of the tree halfway to the adjoining tree; and (3) in the center of an open area with no trees nearer than 20 to 25 feet, and those only small ones. Only an occasional tuft of bunch grass formed the ground vegetation. The soil was Merrimac coarse sand.

The data are shown graphically in figure 1, in which the points of identical moisture content are connected by continuous lines.

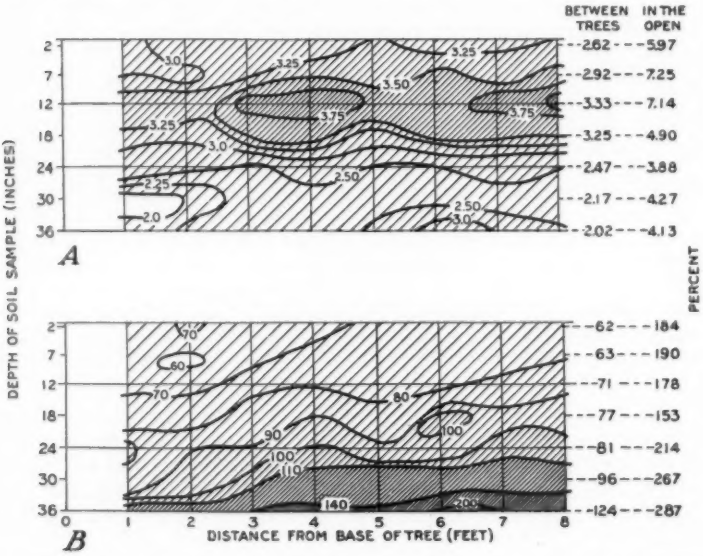

FIGURE 1.—Moisture distribution under a red pine tree having a crown limit of about 4 feet, between trees and in the open, at Rainbow Plantation, July 22, 1930: *A*, Moisture content of soil; *B*, relative wetness.

The greatest amount of moisture is found between the seventh and eighteenth inch layers, centering about 12 inches deep. For the most part there is less moisture at 3 feet than there is at 2 inches. No observations were made on the root system.

When the moisture equivalent is taken into consideration, using the value "relative wetness" (moisture ÷ moisture equivalent × 100) as proposed by Conrad and Veihmeyer,[3] one finds a progressively increasing relative wetness with increases in distance and depth from the base of the tree. The influence of the roots extends beyond the 8-foot line, since both moisture content and relative wetness are much higher at greater distances out in the open.

The moisture present in the soil does not represent the difference between the amount utilized by the tree and the amount originally present in the spring for it was frequently replenished during the

[3] CONRAD, J. P., and VEIHMEYER, F. J. See footnote 2.

growing season by rains. Furthermore, the crown of the tree intercepts a certain amount of rain. The distribution of precipitation that actually reaches the ground under a tree is apt to be quite irregular, and is governed to a considerable degree by the direction from which the wind blows and the intensity and amount of precipitation. No attempt was made to study this question until the summer of 1933, when tin pails 6½ inches in diameter and 4½ inches deep were placed under several different trees at various distances from the base. For several weeks, the amount of water which fell into each bucket was measured immediately after every rain.

The data most comparable to the conditions of this first experiment were obtained under a white pine of somewhat larger size (18-foot crown) on the station grounds. The results are given in table 1.

TABLE 1.—*Amount of precipitation reaching the ground under a white pine tree, 8 inches d.b.h. and 18 feet crown spread, Connecticut Agricultural Experiment Station grounds, summer 1933*

Date	Rainfall	Percentage [1] of rainfall that reached the ground at indicated distance from base of tree			
		0 feet	4.5 feet	6 feet	9 feet
	Inches	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 17	0.043			0	
Aug. 18	.32			67	
Aug. 22	1.93			51	
Aug. 24	.90	121	75		129
Aug. 29	.85	53	75		90
Sept. 4	.59	36	23		115
Average [2]		74.9	61.7	52.3	111.2

[1] Rainfall in the open was regarded as 100 percent.

[2] Average = $\frac{\text{total collected under tree}}{\text{total collected in open}} \times 100$. This applies also to the succeeding tables.

Next to the trunk there is apt to be a concentration of moisture caused by some of the rain running down the branches and trunk. Another point of concentration is at the crown limit, where in this case more rainfall was recorded than in the open. This is due to the rain beating against the edge of the crown and then falling to the ground, and occurs, of course, only on the windward side of the tree when there is considerable horizontal air movement. A drooping of the branches also tends to conduct the water outwards.

Büsgen and Münch [4] quoted figures from Riegler (shown in table 2) on the distribution of a rainfall of 402.8 mm under the crowns of 55-year-old trees. They state that of the summer rainfall, 80 percent reaches the ground under hardwoods and less than 70 percent under conifers. At least 30 percent evaporates from the soil and soil covering. Thus, at most, only half of the rain which falls is absorbed by the roots.

[4] BÜSGEN, M., and MÜNCH, E. THE STRUCTURE AND LIFE OF FOREST TREES. English transl. by T. Thomson. 436 pp., illus. New York.

TABLE 2.—*Figures from Riegler on the distribution of a rainfall of 402.8 mm under the crowns of 55-year-old trees of different species*

Species	Percentage of total precipitation that—			
	Evaporated from crown	Ran down stem	Fell through crown	Reached the soil
	Percent	*Percent*	*Percent*	*Percent*
Beech	21.8	12.8	65.4	78.2
Oak	20.7	5.7	73.6	79.3
Maple	22.5	5.9	71.5	77.5
Spruce	58.8	1.4	39.8	41.2

Hirata,[5] studying the problem in Japan, states that about 32 percent is intercepted in a single rainfall, and 20 percent in a season for all kinds of trees. Most conifers intercept more rain than do hardwoods.

EXPERIMENT 2

Experiment 2 was made under a black oak, *Quercus rubra*, on the Wallingford sand plain, August 5, 1930. This tree, 17 to 18 inches d.b.h. stood alone in a line of small gray birch, probably at one time a fence row. Samples were taken (1) at 2-foot intervals up to 8 feet, and then at 3-foot intervals up to 41 feet, and at depths of 2, 7, 12,

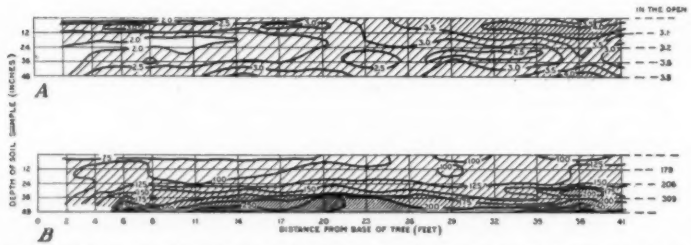

FIGURE 2.—Moisture distribution under a black-oak tree having a crown limit about 20 feet, and in the open, on the Wallingford sand plain, August 5, 1930: *A*, Moisture content of soil; *B*, relative wetness of soil.

18, 24, 30, 36, and 48 inches; (2) at similar depths in the open southwest of this tree with no tree or shrub closer than 50 to 55 feet. The soil, a Merrimac coarse sand, was practically bare of ground vegetation. No root studies were made in this experiment.

The data, given in figure 2, reveal in general the same condition as was found under the pine, although the increase in relative wetness was not so definite. Even a distance of 41 feet from the tree base was not sufficient for the soil to be free from the effects of root absorption. While the percentage of moisture in the open was not higher than it was under the tree, the relative wetness was considerably higher.

Studies relative to the amount of precipitation reaching the ground under this tree were made during the summer of 1933. The data in table 3 show a considerable variation, both between the several posi-

[5] HIRATA, T. CONTRIBUTIONS TO THE PROBLEM OF THE RELATION BETWEEN THE FOREST AND WATER IN JAPAN. Internatl. Cong. Forestry Expt. Stas., Stockholm, Proc. pp. 599–605. 1929.

tions under the tree and between rainstorms. In general, the heavier the precipitation the larger the proportion that finds its way through the foliage to the ground. Instances of quantities in excess of 100 percent can be accounted for by drippings from foliage and branches, the amount depending upon the direction and velocity of the wind. In other words, drippings probably occur with every rain of any consequence, but their point of seepage through the tree and the amount that comes through depend upon the intensity and direction of the storm.

TABLE 3.—*Amount of precipitation reaching the ground under a black-oak tree, 17 to 18 inches d.b.h and of about 40 feet crown spread, on Wallingford sand plain, 1933*

Date	Total rainfall	Percentage [1] of total rainfall that reached the ground at indicated distance from base of tree					
		0 feet	4 feet	8 feet	12 feet	16 feet	20 feet
	Inches	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 20	0.86	----------	78	74	75	80	59
Aug. 22	1.56	----------	70	70	75	72	42
Aug. 23	.06	----------	15	29	49	44	81
Aug. 24	.85	----------	86	84	106	108	102
Aug. 28	.19	63	63	50	67	65	94
Sept. 3	1.07	39	54	64	76	69	----------
Average	----------	43	70	71	78	79	64

[1] Fall in the open was regarded as 100 percent.

EXPERIMENT 3

Experiment 3 was made under Corsican pine, *Pinus laricio*, in block 45, Rainbow plantation, July 15, 1932, only a few hundred feet from the red pine sampling of 1930. These trees in this block are among the few occasional survivals of a planting made in 1910, which for the most part was a failure. Beginning on a line between two trees, the sampling was as follows: (1) Out in the open in a northwest direction at 2, 4, 6, 8, 10, 12, 14, 20, and 23 feet and at depths of 2, 6, 12, 18, 24, 36, and 48 inches; (2) starting at the same point but in the opposite direction, at 2, 4, 11, and 15 feet. In this case there were additional trees on one side of the trench and also a pair of trees 3 feet beyond the 15-foot sampling. The upper part of figure 3, *A*, shows a ground plan of the points of sampling in relation to the position of the trees. The pair of trees beyond the 15-foot sampling is not shown. The trees were approximately 17 to 20 feet tall at the time this study was conducted.

Figure 3, *B* and *C*, shows a very good correlation between the zones of lower moisture content and their proximity to the trees involved, this being true both in actual moisture content and in relative wetness.

Notes taken of the root distribution as observed along the walls of the trench indicate that the bulk of the roots were confined more or less to two general levels, 1 to 7 inches and 18 to 28 inches, and were present at all distances from the trees, being, of course, fewer and smaller at the greater distances. The relative wetness values in figure 3 show a fairly definite relation to the presence of the upper strata of roots but not to the lower strata.

EXPERIMENT 4

Experiment 4 was made under a black oak on the Wallingford sand plain, August 3, 1933, about 100 yards southeast of the 1930 sampling. This was a lone tree with no vegetation within 35 feet, except for an occasional tuft of bunch grass. The sampling was as follows: (1) Starting 30 feet southwest of the tree, a hole was dug 4 feet deep with a 2-foot face parallel to the crown of the tree on that side. The approximate size and position of all roots visible in the face of the pit were plotted and samples for moisture were taken from the face of the pit at depths of 2, 6, 12, 18, 24, 36, and 48 inches. Digging then proceeded 3 feet more towards the tree, and observations were made on the new face of the elongated pit. This was continued up to within 3 feet of the trunk. (2) Starting 18 feet northwest of the same tree, the procedure was duplicated, except that 24 inches was the limit of depth.

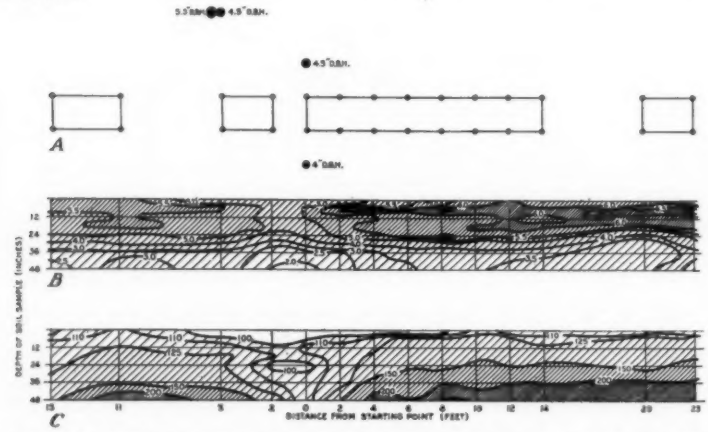

FIGURE 3.—Moisture distribution under Corsican pine having a crown limit of about 4½ feet, Rainbow plantation, July 15, 1932: *A*, Ground plan of trench and sampling points with respect to the trees (a pair of trees 3 feet from the left end of the trench is not shown, although the effect of their presence is indicated by the graphs); *B*, moisture content of soil; *C*, relative wetness of soil.

The data are presented in figure 4. Both the moisture-content and the relative-wetness charts show the effect of the tree roots on the soil moisture. The sampling on the northwest side is particularly good in this respect.

In figure 4, *B*, each block represents a cross section of the trench at the corresponding distance from the tree. These studies indicate a fairly equal distribution of tree roots above the 27- or 28-inch level, with practically none below that depth. The larger roots tend to be concentrated between the 12- and 24-inch levels. While it is difficult to make any direct comparison between presence of roots and soil-moisture content without actually removing and weighing the roots yet a general relationship is ascertainable—the relative wetness tending to be greater where the roots are fewer.

In table 4 is shown the relative amount of precipitation that reached the ground under this tree. As in the cases previously given, the amount varied considerably with the individual rains and with the

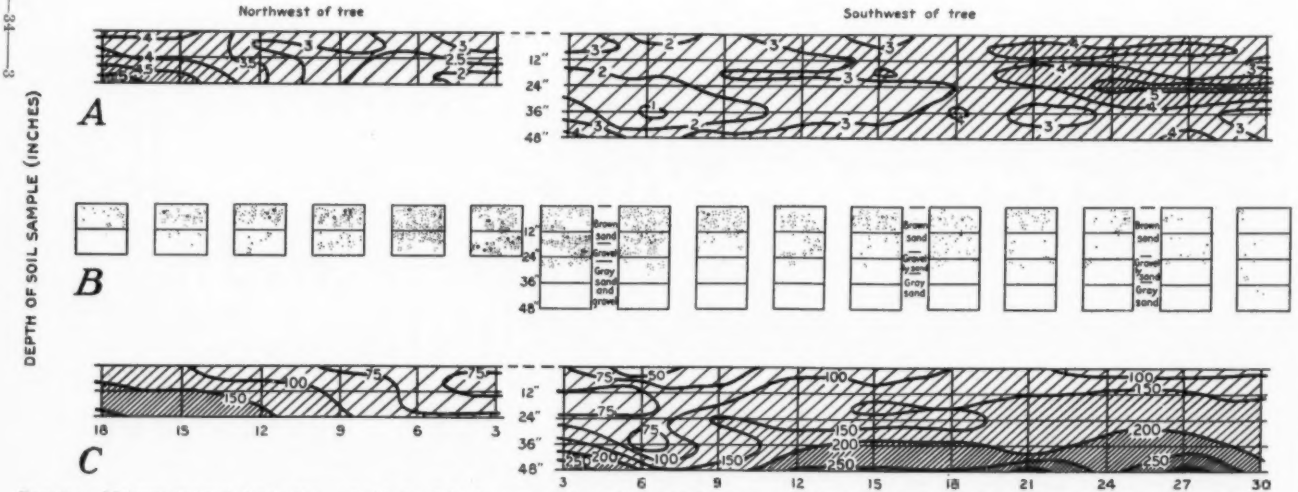

FIGURE 4.—Moisture and root distribution under black oak having a crown limit of 9 feet, Wallingford sand plain, August 3, 1933. *A*, Moisture content of soil. *B*, Root distribution. The roots are not drawn to scale, most of the smaller ones being only 1 to 2 mm in diameter. *C*, Relative wetness of soil.

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different positions under the tree. In these rainfall studies under oak, the buckets were placed along the line of the previously existing trench from which the soil samples had been taken.

TABLE 4.—*Amount of precipitation reaching the ground under a black oak tree, 11 inches diameter at about 1 foot and crown spread about 17 feet, Wallingford sand plain*

Date	Total rainfall	Percentage [1] of total rainfall reaching ground from indicated direction and distance from base of tree							
		Northwest				Southwest			
		9 feet	6 feet	3 feet	0 feet	0 feet	3 feet	6 feet	9 feet
	Inches	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 20	0.86	99	99	82	83	60	83	114	116
Aug. 22	1.56	102	113	101	67	31	86	64	70
Aug. 23	.06	65	3	0	0	12	7	7	32
Aug. 24	.85	57	47	50	36	55	70	87	103
Aug. 28	.19	100	82	31	28	30	37	71	98
Sept. 3	1.07	69	54	41	27	52	75	147	108
Average		85	82	70	53	46	77	[2] 81	95

[1] Rainfall in the open was regarded as 100 percent.
[2] Average of first 5 dates only.

DISCUSSION

Three factors are recognized as influencing the moisture content of the soil after any given rainfall in midsummer, namely, surface evaporation, interception by the crown, and absorption by the roots. The low relative wetness values found close to the surface in all cases was due, of course, to surface evaporation. Interception was of less consequence in the case of these trees growing in the open than it would be in a dense forest. Nevertheless these studies have shown that immediately beneath the crown only about 75 percent of the total precipitation reaches the ground. Here a proper evaluation of the two forces involved—interception and utilization—is difficult without further rather extensive experimentation.

Beyond the crown limit and below the immediate surface, absorption by the roots is the principal force tending to lower the moisture content of the soil. These studies show that such absorption does take place. Although the observations on root development were rather limited, they were sufficient to indicate that the region of moisture removal coincides in a general way with the area occupied by the roots. In other words, the whole root system is involved in moisture absorption and not any one particular portion. This is not true of nutrient absorption, since the bulk of the available plant nutrient supply, particularly nitrogen, is to be found comparatively close to the surface, and therefore most of the feeding roots are located in that portion of the profile. This is particularly true in northern forests where there is a considerable accumulation of duff on the forest floor. In other words, the tree may obtain most of its nutrients from the surface 10 or 12 inches, but except in wet soils it must draw also upon the subsoil to meet its moisture needs.

SUMMARY

This study was made to determine the actual distribution of soil moisture under individual trees in the open during a dry period in summer. In practically all cases the lowest moisture content was found immediately beneath the crown close to the base of the tree at two depths—the soil surface, and between the second and fourth foot. The highest moisture content was usually at about the 1-foot level, and increased with distance from the tree.

Relative wetness values were lowest in the immediate vicinity of the tree base, and they increased in irregular zones with increased distance from the trunk, and with depth. In other words, while the highest percentage of moisture in the upper 4 feet of soil was found at about 1 foot, the highest relative wetness values were found at the 3- or 4-foot levels.

THE INHERITANCE OF QUALITY IN TRUMBULL AND MICHIKOF VARIETIES OF WINTER WHEAT[1]

By W. W. WORZELLA[2]

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INTRODUCTION

Economic wheat breeding usually includes the improvement of quality as one of its chief aims. The term "quality" though relative includes many factors. Of these probably the most important is gluten strength or baking strength as it is often referred to. It is in this sense only that the term "quality" is used in this paper.

In order to effectively pursue a breeding program by which improved varieties of wheat can be produced, plant breeders should have a knowledge of the manner of inheritance of the characters concerned. While much progress has been made in the breeding of wheat of improved quality, it has been accomplished in spite of the fact that the inheritance of gluten strength has not been fully understood. This lack of understanding has doubtless been due, in part at least, to the absence of a suitable technic for measuring gluten strength as well as to the fact that this characteristic is much influenced by environmental conditions. This paper reports a study of the inheritance of gluten quality when evaluated by the wheat-meal fermentation time test. It was carried out on winter wheat hybrids grown at Lafayette, Ind., in 1931–32 and 1932–33.

REVIEW OF LITERATURE

Many reports have been published on quality in wheat, but few data are available on the inheritance of this character. Most workers have investigated it largely from the standpoint of kernel texture or crude protein content of the grain.

Biffen (*1*, *2*)[3] and Howard and Howard (*13*) presented evidence to show that hardness and softness of endosperm behave as a simple Mendelian character.

Baking strength appeared to Saunders (*16*, *17*) as being complex and probably depending on a number of Mendelian characters working together.

Clark (*3*), Clark and Hooker (*4*), and Clark, Florell, and Hooker (*5*) studied the inheritance of quality in wheat hybrids, using crude-protein content and kernel texture as indices. They concluded that these behave as quantitative characters; however, the number of genetic factors involved could not be determined.

Hayes, Immer, and Bailey (*12*) computed interannual correlation coefficients for various quality indices and found a general tendency

[1] Received for publication Mar. 21, 1934; issued November, 1934. Based upon part 1 of a thesis submitted to the faculty of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy, June 1934.

[2] The writer wishes to express his appreciation to Dr. G. H. Cutler, assistant chief and professor of agronomy, for his advice and interest during the course of these investigations; to Mrs. W. W. Worzella for her help in making the fermentation tests; to S. R. Miles, assistant in agronomy, for suggestions given in the statistical interpretation of the results; and to the National Milling Co. for financial assistance.

[3] Reference is made by number (italic) to Literature Cited, p. 713.

toward a correlation between the results of different seasons; however, some correlations were negative and others were not statistically significant.

Cutler and Worzella (7), using "time" as an index of quality, obtained positive interannual correlations of high magnitude between results of different seasons.

MATERIALS AND METHODS

MATERIALS

The experiments here reported were conducted with F_1, F_2, F_3, and parent plants grown under field conditions during 1931–32 and 1932–33. During this period the quality of over 6,000 samples of wheat, each sample representing the seed from an individual plant, was determined by the fermentation test. Since all of the plants did not produce sufficient seed to permit a duplicate test on each sample, about 10,500 individual time tests were made.

The two varieties used as parents in this study, Trumbull and Michikof, are both winter wheats. Extensive milling and baking tests, as well as the wheat-meal fermentation time test (*6*, *7*), have shown that Trumbull has a weak gluten, while the gluten of Michikof is strong.

Crosses between Trumbull and Michikof were made in the greenhouse during the winter of 1929–30. The F_1 plants were grown in the greenhouse during 1930–31. Additional crosses were made later to determine the quality of F_1 kernels and to grow several F_1 plants in the field during 1932–33.

The F_2 generation was grown in the field at the soils and crops farm during 1931–32. The kernels were spaced at 6-inch intervals in 25-foot rows 1 foot apart. Checks of the parent varieties, sown in the same manner, were alternated every seventh row.

A random selection of the F_2 population was made as a basis for continuing the study in the F_3 generation. In the fall of 1932, 50 grains from each F_2 plant were used to seed an F_3 progeny row by spacing the kernels at 6-inch intervals in 25-foot rows 1 foot apart. A few rows were similarly seeded with F_2 kernels and one was seeded with F_1 kernels. Check rows of the parents were alternated every fifth row throughout the plot.

METHODS

The gluten strength of the seed of individual plants was determined by the wheat-meal fermentation time test. Since the details of the fermentation test have previously been reported by Cutler and Worzella (*6*, *7*), only a brief description of the method is given here.

Eight grams of sound, clean, normal wheat was ground to the desired fineness on the Labconco mill. To each 3.5-g sample of the meal, 2 cc of 10-percent Fleischmann's yeast suspension was added, and with a glass stirring rod mixed into a mass. The mass was then transferred to the palm of the left hand and with the right thumb was kneaded until it became a dough ball of medium-stiff consistency. The dough balls were then placed in 50-cc beakers containing 30 cc of distilled water kept at 80° F. (26.7° C.). The time was recorded at once and the beakers placed in a fermentation cabinet maintained at 80°. Regular observations were made of all dough balls to observe

when disintegration began. When this took place the time was again recorded. The time of each sample thus obtained was recorded in minutes, and consisted of the difference between the original immersion and the first disintegration of the dough. Thus time became the index or measure of quality. Wheats with a long time possess strong gluten, while those with a short time have a weak gluten.

Since, in this paper, time is used as an index or measure of gluten strength, it appears necessary to establish its reliability by determining the relationship of time to other indices commonly used in estimating quality. Cutler and Worzella (*6*, *7*) compared time, as measured by the fermentation test, with other indices used in estimating quality, on 309 wheat samples covering 4 years' investigations, and obtained significant positive correlations between time and protein content, loaf volume, absorption, and percentage of vitreous kernels, respectively. Wilson, Markley, and Bailey (*18*) obtained positive correlation coefficients between time and loaf volume and strength score in wheat varieties carefully selected for protein content. Pelshenke (*14*, *15*), using essentially the same technic, reported a good agreement between time and baking tests, as well as the quality numbers obtained with the Brabender farinograph, and the swelling methods of Berliner and Koopman, respectively. Hauser (*11*) obtained significant positive correlations between time and the "curve area of softening" and dry gluten content, respectively. Winter and Gustafson (*19*) presented data that show positive correlations between time and protein content as well as loaf volume. Edel (*8*) determined the quality of over 2,000 samples of wheat by means of the fermentation test, and concluded that in 80 percent of the cases the results obtained were sufficiently reliable for the milling trade. In the light of the foregoing evidence it appears that time as measured by the fermentation test, can be used as a reliable measure of gluten strength.

In preparing a frequency table, with time in minutes as the index of quality, a constant percentage was used in calculating class intervals. This was necessary because time has a logarithmic relationship, that is, an increase in minutes represented by the change from 50 to 100 is as important as an increase from 100 to 200. For example, assuming 10 percent variability below the mean, a strain of wheat having an average time of 30 minutes would vary from 27 to 33.3 minutes, while another strain with an average time of 300 minutes would vary from 270 to 333 minutes. In percentage, both varied 10 percent; however, in actual minutes the first strain had a range of only 6.3 minutes, while the second had a range of 63 minutes. Because a constant percentage change is also a constant logarithmic change, all calculations were made in logarithms and later changed to antilogarithms. In order to divide the range of variation into seven classes, a logarithm of 0.132 was used in calculating class intervals.

In order to compare the variability of populations with widely different means, it is necessary to use relative rather than absolute variabilities. As a rule the coefficient of variability is used, but its application in this paper is modified somewhat, since by the use of logarithms, the percentage variability becomes greater above the mean than below when the mean is used as the base in both cases. For example, if an assumed logarithm of 2.0 is used as the mean and the logarithm 0.038 is used as one standard deviation, it is expected

that the chances are 2 to 1 that the true value of the mean logarithm is between 1.962 and 2.038. The corresponding antilogarithms are 91.6 and 109.1, respectively. These values are 8.4 percent below and 9.1 percent above the mean value 100. As used in this paper, the coefficient of variability is the percentage variability below the mean, which is 8.4 percent in the example cited above. Ezekiel (*9, p. 116*) treats a somewhat similar problem.

EXPERIMENTAL RESULTS

RESULTS OF 1931–32

Quality of F_1 Endosperm

In order to determine the quality of F_1 seeds, crosses were made under field conditions between Trumbull and Michikof and the reciprocal. For direct comparison with F_1 seeds, the kernels of the parents were obtained from heads that had been emasculated and pollinated in the same manner as the heads containing the hybrid seeds. The data obtained are shown in table 1.

Table 1.—*The quality of parent and F_1 seed in Trumbull × Michikof and reciprocal cross in 1932*

Parent or cross	Time	
	Actual	Expected
	Minutes	*Minutes*
Trumbull	55	55
T ♀ × M ♂ (F_1 seed)	85	[1] 93
M ♀ × T ♂ (F_1 seed)	199	[2] 157
Michikof	266	266

[1] (log. 1.7404+1.7404+2.4249) ÷3=1.9686=93. [2] (log. 1.7404+2.4249+2.4249) ÷3=2.1967=157.

Since the F_1 endosperm is the result of double fertilization, the quality of the F_1 seed would depend on which way the cross was made, because the female contributes factors in two nuclei in the formation of the endosperm, while the male contributes factors in only one nucleus. It will be noted from the data presented in table 1 that with Trumbull as the female parent, a time of 85 minutes was obtained, whereas with Michikof as the female parent the time was 199 minutes. The actual results obtained agree closely with those expected, considering that only single tests were made.

Quality of Grain of F_2 Plants

To study the inheritance of quality during 1931–32, fermentation tests were made on the grain obtained from the F_2 plants as well as from the parental plants. The data are shown in table 2.

Table 2.—*Frequency distribution of time for quality in cross of Trumbull and Michikof wheat in 1931–32*

Parent or cross	Plants in class center for time in number of minutes stated									Plants	Mean	Coefficient of variability
	32.0	43.3	58.7	79.5	107.8	146.0	197.9	268.1	363.4			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Minutes*	
Trumbull	72	24								96	34.5	12.46
Michikof								34	89	123	334.2	12.72
F_2 plants	6	88	107	124	120	153	267	191	20	1,076	131.8	45.22

The results show that the average time of disintegration for dough balls made from Trumbull wheat is 34.5 minutes, and from Michikof 334.2 minutes, with coefficients of variability of 12.46 and 12.72, respectively. This indicates that both parents though widely different are homozygous for gluten strength. The gluten strength of the F_2 plants varied all the way from that of the weak to that of the strong parent. The hybrids, considered as a whole, were about intermediate to the parents, with an average time of 131.8 minutes. There is, however, a preponderance of hybrids toward the long time side of the frequency distribution which may indicate partial dominance of strong gluten, or express the effect of environmental conditions favoring the development of strong gluten wheat. The sharp rise in variability between the parents and the F_2 generation, and the reappearance of both parental types, strongly suggest the existence of segregation of genetic factors.

RESULTS OF 1932–33

The quality of the grain from individual plants in the F_1, F_2, and F_3 generations of a Trumbull×Michikof cross and of the parent varieties, was determined when grown under similar environmental conditions. The data for these and also for several selected F_3 families are presented in table 3.

TABLE 3.—*Frequency distribution of time for quality in the F_1, F_2, and F_3 generations of Trumbull×Michikof cross and of parent varieties in 1932–33*

Parent or cross	Plants in class center for time in number of minutes stated								Plants	Mean	Coefficient of variability
	43.3	58.7	79.5	107.8	146.0	197.9	268.1	363.4			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Minutes*	
Trumbull	68	214	40						322	57.1	15.59
Michikof						10	226	51	287	280.0	12.46
T♀×M♂ } F_1 plants				3	26				29	141.2	9.14
M♀×T♂ } F_1 plants				1	6				7		
F_2 plants		16	37	81	120	92	18	3	367	138.3	31.09
F_3 families		1	16	28	45	40	16	1	3,556	149.7	30.66
Pedigree no.:											
5–44	4	20	1						25	56.6	12.19
5–51	1	10	16	3					30	72.6	18.87
5–32		1	5	9	8	1			24	111.9	24.49
2–65		1	4	4	8	5	2		24	135.3	32.59
5–30				2	17	7			26	154.8	15.63
8–22				1	8	12	6		27	189.1	21.63
2–11						3	17	8	28	283.1	16.67
1–23							6	16	22	334.5	12.56

The average time of disintegration for dough balls made from Trumbull wheat was 57.1 minutes and for Michikof 280 minutes. The F_1 plants (F_2 endosperm) were intermediate to the parents in gluten strength. The results show that in the F_2 generation, as a whole, the hybrids are intermediate to the parents in gluten strength; however, both parental types were recovered. F_3 families just as weak as Trumbull and just as strong as Michikof in gluten strength were obtained although only 147 families were studied. The gluten strength of all F_3 families taken together show an average time of 149.7 minutes with a coefficient of variability of 30.66. Some of the F_3 lines (5–44 and 1–23) bred comparatively true, the range for time being no greater than for the parents, while others varied as much as the F_2 generation. The great increase in the variability between

F_1 and the F_2 or means of F_3 families, and the reappearance of both parental types in later generations indicates clearly the existence of segregation of genetic factors.

GENETIC INTERPRETATION OF THE RESULTS

F_3 populations were grown and tested for quality because of the greater reliability of data based on the breeding behavior of F_3 progenies than on that of F_2 plants alone. The quality of a given F_2 plant may be greatly influenced by environment, but a whole row of plants from seeds of such an F_2 would hardly be influenced to the same extent. The F_3 data, representing 147 families or 3,556 plants, are used in the genetic interpretation of these results. Coefficients of variability were calculated for each F_3 progeny so that the comparative sizes of these coefficients could be used as an aid in estimating the number of genetic factors involved. Table 4 shows the distribution of parental rows as well as the 147 F_3 families according to time and coefficients of variability.

Data from 15 to 35 plants for each row were used in calculating the means and coefficients of variability. An inspection of table 4 shows that the coefficients of variability in the different F_3 families ranged from 7 to 37 percent.

Since parental recombinations as to quality reappeared and were apparently homozygous in the F_3 generation, when only 147 families were studied, it appeared probable that 3 or 4 factor pairs were involved in the inheritance of gluten strength. If 3 factor pairs were involved, parental recombinations would be expected to appear once in every 64 families; with 4 factor pairs, only once in 256 families. Since a poor fit was obtained with 4 factor pairs, it seemed probable that 3 factor pairs would explain the difference between the two varieties. Accordingly, the F_3 lines, which were classified into seven groups, were compared with the 1:6:15:20:15:1 genotypic ratio which is expected when three independent factor pairs are involved. The observed and calculated numbers, compared by the χ^2 method, are shown in table 5.

TABLE 4.—*Frequency distribution of the Trumbull and Michikof parents and of F_3 families arranged in classes according to the mean time and coefficient of variability*

Parent or cross	Rows or families in class center for time in number of minutes stated							Centers of coefficient of variability classes	Rows or families
	58.7	79.5	107.8	146.0	197.9	268.1	363.4		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Michikof						6	1	7	15
						7	1	10	
	2							10	
Trumbull	9							13	14
	3							16	
					2			7	
	1	1		3	7	5	1	10	
		5	2	4	6	4		13	
		3	9	9	6	5		16	
		6	3	7	7	2		19	147
F_3 families		1	4	5	7			22	
			6	10	3			25	
			4	3	2			28	
				2				31	
				1				34	
				1				37	

TABLE 5.—*Segregation of 147 F_3 families of Trumbull and Michikof crosses for quality, compared by the χ^2 method with a calculated 1:6:15:20:15:6:1 genotypic ratio*

Class center for time (minutes)	F_3 families		$O-C$	$\frac{(O-C)^2}{C}$	Class center for time (minutes)	F_3 families		$O-C$	$\frac{(O-C)^2}{C}$
	Observed	Calculated				Observed	Calculated		
	Number	*Number*				*Number*	*Number*		
58.7	1	2.297	−1.297	0.7323	197.9	40	34.453	5.547	0.8931
79.5	16	13.781	2.219	.3573	268.1	16	13.781	2.219	.3573
107.8	28	34.453	−6.453	1.2086	363.4	1	2.297	−1.297	.7323
146.0	45	45.937	−.937	.0191					

NOTE.—$\chi^2=4.30$. $P=0.64$.

According to Fisher's (*10*) table, $P=0.64$, which indicates that 64 times in 100 trials a poorer fit than the one obtained would be expected as a result of chance alone. The following hypothesis is, therefore, suggested to explain the observed segregation of quality. Assuming that three independent factor pairs are involved in the inheritance of gluten strength, then the weak gluten parent, Trumbull, may be considered to have the genotype $s_1s_1\ s_2s_2\ s_3s_3$, and the strong gluten parent, Michikof, the genotype $S_1S_1\ S_2S_2\ S_3S_3$. Let it be further assumed that each factor has a cumulative effect, so that each capitalized symbol (S) represents an increment of gluten strength when combined with the weak gluten parent. The F_1 plants (F_2 endosperm) would be $S_1s_1\ S_2s_2\ S_3s_3$, and intermediate to the parents in gluten strength. The F_2 genotypes and their breeding behavior in the F_3 generation would be as shown in table 6.

An inspection of table 6 shows that, theoretically, some F_3 progenies should breed true for quality, while others would segregate for 1, 2, or 3 genetic factor pairs. Referring again to table 4, it will be noted that some F_3 families bred true for quality, since their coefficient of variability was no greater than that of the parents. Other families showed coefficients of variability ranging from 7 to 37 percent, depending upon whether 1, 2, or 3 genetic factor pairs were segregating for gluten strength. While clear-cut classes of coefficients of variability cannot be definitely established from the data, it can be determined from the relative increases in the coefficients of variability in the different groups whether a family is homozygous or segregates for one or more genetic factors.

In general, it seems clear that the three-factor hypothesis here advanced satisfactorily explains the genetic behavior of the varieties of wheat studied. Nevertheless, it will be remembered that there are varieties of wheat that are weaker in gluten strength than Trumbull and others that are stronger than Michikof. This suggests that there are probably more than three factors involved in the inheritance of quality; however, this does not invalidate the three-factor hypothesis that has been assumed to explain the genetic difference between the Trumbull and Michikof varieties. Genetic studies with other parental varieties will be necessary to determine the probable number of factors that condition the mode of inheritance of quality in wheat. The writer is not unmindful, however, of the fact that such a definite interpretation may not always hold true, since environment greatly influences the expression of the quality character.

TABLE 6.—*F_2 genotypes and their breeding behavior in the F_3 generation, with the average number of increments (S) and average quality for each group*

Number of increments (S)	F_2 genotype	Parts of 64	F_3 breeding behavior	Class centers for time
				Minutes
6	S_1S_1 S_2S_2 S_3S_3	1	Breeds true	363.4
5	S_1S_1 S_2S_2 S_3s_3	2	Segregates 1:2:1	268.1
	S_1S_1 S_2s_2 S_3S_3	2	do	
	S_1s_1 S_2S_2 S_3S_3	2	do	
4	S_1S_1 S_2S_2 s_3s_3	1	Breeds true	197.9
	S_1S_1 s_2s_2 S_3S_3	1	do	
	s_1s_1 S_2S_2 S_3S_3	1	do	
	S_1S_1 S_2s_2 S_3s_3	4	Segregates 1:4:6:4:1	
	S_1s_1 S_2S_2 S_3s_3	4	do	
	S_1s_1 S_2s_2 S_3S_3	4	do	
3	S_1s_1 S_2s_2 S_3s_3	8	Segregates 1:6:15:20:15:6:1	146.0
	S_1S_1 S_2s_2 s_3s_3	2	Segregates 1:2:1	
	S_1s_1 S_2S_2 s_3s_3	2	do	
	S_1s_1 s_2s_2 S_3S_3	2	do	
	S_1S_1 s_2s_2 S_3s_3	2	do	
	s_1s_1 S_2S_2 S_3s_3	2	do	
	s_1s_1 S_2s_2 S_3S_3	2	do	
2	S_1S_1 s_2s_2 s_3s_3	1	Breeds true	107.8
	s_1s_1 S_2S_2 s_3s_3	1	do	
	s_1s_1 s_2s_2 S_3S_3	1	do	
	S_1s_1 S_2s_2 s_3s_3	4	Segregates 1:4:6:4:1	
	S_1s_1 s_2s_2 S_3s_3	4	do	
	s_1s_1 S_2s_2 S_3s_3	4	do	
1	S_1s_1 s_2s_2 s_3s_3	2	Segregates 1:2:1	79.5
	s_1s_1 S_2s_2 s_3s_3	2	do	
	s_1s_1 s_2s_2 S_3s_3	2	do	
0	s_1s_1 s_2s_2 s_3s_3	1	Breeds true	58.7

INTERANNUAL CORRELATIONS

Interannual correlation coefficients were calculated to determine to what extent the quality of the F_2 and F_3 generations agreed. When the time of 147 F_2 plants was correlated with that of the means of the F_3 families, a correlation coefficient of $+0.842 \pm 0.024$ was obtained. This indicates that the gluten quality of individual plant selections in the early segregating generations, as determined by the fermentation test, may serve as a dependable guide in making selections for quality.

Since time is used as a basis of selection for quality in the wheat-breeding program in the agronomy department, a large number of pure-line and hybrid strains grown in the wheat-breeding nursery have been tested for gluten strength during the last 3 years. Interannual correlation coefficients were calculated for a few of these strains to determine the extent to which they would react the same in different seasons. The data for the pure-line strains and hybrid selections are given in table 7.

TABLE 7.—*Interannual correlation coefficients in soft winter wheat hybrid selections and pure-line strains in the harvest seasons of 1931, 1932, and 1933*

Designation	Samples	Years correlated	Coefficient of correlation
	Number		
Pure-line strains	43	1931–32	$+0.686 \pm$ [1] 0.082
	43	1932–33	$+.819 \pm$ .051
	43	1931–33	$+.806 \pm$ .054
Hybrid selections	67	1931–32	$+.671 \pm$ .057
	56	1932–33	$+.524 \pm$ .098
	56	1931–33	$+.393 \pm$ .114

[1] Standard errors.

The data show that significant positive correlations were obtained in all cases when time of a given group of wheats in one year was correlated with time for the same wheats in another year. These results present additional evidence that quality is an hereditary quantitative character, and is apparently accurately measured by the fermentation test.

SUMMARY

The inheritance of quality, as determined by the fermentation test, was studied on the progenies involving a cross between Trumbull, a weak gluten parent, and Michikof, a strong gluten parent, when grown under field conditions during 1931–32 and 1932–33.

The quality of over 6,000 samples of wheat, each sample representing the seed from individual F_1, F_2, F_3, and parent plants, was determined.

The gluten strength of the F_1 seed was weak when Trumbull was used as the female parent and strong when Michikof was used as the female parent. The F_1 plants (F_2 endosperm) were intermediate to the parents in quality.

In the F_2 generation, the hybrids were intermediate in gluten strength; however, plants varied all the way from the weak to the strong gluten parent.

Considered as a whole, the F_3 families were intermediate to the parents in quality. Families as weak as Trumbull and as strong as Michikof were obtained. Some bred true while others showed coefficients of variability ranging from 7 to 37 percent, depending on whether one or more genetic factors for gluten strength were segregating.

A genetic interpretation of the results is given by assuming that, in the varieties of wheat studied, quality is a quantitative character governed by three major independent factor pairs. Trumbull was considered to have the genotype $s_1s_1\ s_2s_2\ s_3s_3$ while Michikof had the genotype $S_1S_1\ S_2S_2\ S_3S_3$. The observed and calculated numbers, compared by the χ^2 method gave a P value of 0.64.

Correlating time of the F_2 and F_3 data resulted in a coefficient of $+0.842 \pm 0.024$, indicating that the gluten quality obtained in the early generations can serve as a dependable guide in making selections for quality.

Significant positive interannual correlation coefficients for time were obtained between the results of different seasons when wheats of diverse nature were grown under similar environmental conditions. These results present additional evidence that gluten strength is an hereditary quantitative character and is apparently accurately measured by the fermentation test.

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EFFECT OF METHOD OF MANUFACTURE ON THE NUTRITIVE VALUE OF FISHMEALS AS DETERMINED BY GROWTH STUDIES WITH CHICKS[1]

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INTRODUCTION

In earlier work on the comparative nutritive value of different fishmeals for chicks at the Ohio station (*8*),[2] it was shown that the meals varied greatly in their ability to induce growth, owing to a difference in the protein value and in the content of the vitamin G complex. Some evidence obtained suggested that the method of manufacture, or the temperature at which the meals were dried, affected their nutritive value. In order to obtain more definite information on the effect that methods of processing and drying temperatures exert on the nutritive value of the meal, the study herein reported was undertaken.

REVIEW OF LITERATURE

That drying methods affect the feeding value of fishmeals has been shown by several investigators. Ingvaldsen (*3*) reported that the method of preparing and drying fish byproducts affected the nutritive value of the resulting meal. Likewise, Daniel and McCollum (*2*), Maynard, Bender, and McCay (*4*), and Schneider (*9*) have presented evidence showing that flame-dried menhaden meals have a lower biological value than steam-dried menhaden or vacuum-dried whitefish meals. Maynard and Tunison (*5*) using rats as experimental animals, made a comparative study of flame and vacuum-dried menhaden and haddock meals, and reported that flame-drying lowered the biological value and digestibility of the proteins in each type of meal, although the haddock meals were superior to the menhaden meals.

MATERIALS AND METHODS

The fishmeals used in the present investigations were prepared under experimentally controlled conditions in the technological laboratories of the Bureau of Fisheries, in Gloucester, Mass.[3] All the meals were prepared either from a part or from the whole waste of the cod or haddock fillet industry. Two methods of processing were used in the preparation of the meals. In one case the whole haddock or cod waste, consisting of the heads, backbones, and tails plus the flesh adhering to these portions after the fish had been eviscerated and filleted, was run through a cutter, cooked with live steam for approximately 20 minutes, centrifuged to remove the water, and then dried at different temperatures. In the other case the haddock waste

[1] Received for publication Apr. 10, 1934; issued November, 1934.
[2] Reference is made by number (italic) to Literature Cited, p. 721.
[3] The meals were prepared under the supervision of R. W. Harrison, of the Bureau of Fisheries, who supplied the data regarding their method of preparation.

was run through a cutter and dried directly, without precooking. The two methods were, in reality, a comparison of the wet-rendering and dry-rendering processes.

Meals 1, 2, 3, 4, and 10 were prepared by the wet-rendering (cooking) process, whereas meals 5, 6, 7, 8, 9, and 11 were dried without cooking and centrifuging (dry-rendered). Samples 2 to 11, inclusive, were dried in a small jacketed dryer equipped with an internal agitating and wall-scraping device. Steam or hot water was used in the jacket. Sample 1 was dried in a small flame dryer, by direct heat furnished by a gas burner. Meal 11 was prepared from haddock viscera practically free from livers, by drying under a vacuum without cutting, cooking, or centrifuging. The essential data regarding the method of preparation of the meals are presented in table 1.

TABLE 1.—*Essential data pertaining to the preparation of the fishmeals*

Meal no.	Kind of fish waste	Part of waste	Process [1]	Vacuum	Source of heat in dryer	Temperature in dryer		Approximate time in dryer
						Initial	Final	
						°F.	°F.[2]	*Minutes*
1	Haddock	Whole	Wet	No	Direct flame	400+	400+	30
2	do	do	do	No	Steam	190	218	330
3	do	do	do	Yes	do	145	165	200
4	do	do	do	Yes	Hot water	105	140	360
5	do	do	Dry	No	Steam	210	212	345
6	do	do	do	Yes	do	140	170	210
7	do	do	do	Yes	Hot water	105	135	480
8	do	Backbones	do	Yes	do	100	135	485
9	do	Heads and tails	do	Yes	do	100	135	390
10	Cod	Whole	Wet	No	Steam	212	220	360
11	Haddock	Viscera	Dry	Yes	do	104	220	400

[1] Process refers to whether the material was cooked and centrifuged (wet) prior to drying or dried directly (dry).

[2] Drying temperature was over 400° F., the limits of the thermometer available.

In all of the experiments white leghorn chicks from the same parent stock were used. They were started on their respective rations shortly after they were taken out of the incubator. All lots were brooded indoors on 2-mesh hardware cloth. Individual chick weights and group feed-consumption records were taken each week. The same management was used in all the experiments.

The basal ration was the same in the several experiments. It consisted of ground wheat, 20 parts; wheat bran, 10; cod-liver oil, 1; salt, 1; fishmeal or meat scraps to furnish 10 percent protein ($N \times 6.25$), and yellow corn to make 100 parts. The protein content of the rations was kept practically constant, varying between 18.5 to 19 percent in the different series of experiments. Either 20 or 25 chicks were started in each lot in a trial. One lot, which received a ration supplemented with meat scraps, was included in each trial as a basis for comparison. Sufficient quantities of the different fishmeals and meat scraps were set aside at the beginning of the investigation to last throughout the different trials. The chemical analyses of the products are given in table 2.

TABLE 2.—*Analyses of the fishmeals and meat scraps used as supplements in the investigations*

Supplement	Moisture	Ether extract	Ash	Protein (N×6.25)	Undetermined
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Haddock meal 1	5.33	1.28	26.77	61.87	4.75
Haddock meal 2	5.41	.82	27.55	63.87	2.35
Haddock meal 3	4.94	.92	25.19	65.75	3.20
Haddock meal 4	7.71	1.06	27.06	60.37	3.80
Haddock meal 5	5.47	.73	25.59	64.43	3.78
Haddock meal 6	7.05	1.37	25.17	61.93	4.48
Haddock meal 7	9.03	.90	24.76	62.12	3.19
Haddock meal 8	9.48	.37	17.42	71.12	1.61
Haddock meal 9	8.84	.53	25.63	61.68	3.32
Cod meal 10	4.53	.86	25.34	67.37	1.90
Viscera meal 11	1.40	19.37	23.50	44.50	11.23
Meat scraps	8.12	8.72	23.42	50.31	9.43

The initial experiments involved a comparison of the different fishmeals with meat scraps, when fed on the same (10 percent) protein basis. Four separate trials were conducted with a total population of 90 chicks on each ration.

TABLE 3.—*Effect of different fishmeals on growth of chicks and the incidence of leg paralysis*

[Average of 4 experiments]

Supplement	Average weight of chicks at 8 weeks			Leg paralysis
	Females	Males	Males and females	
	Grams	*Grams*	*Grams*	*Percent*
Meat scraps	356.0± 9.1	405.7±10.6	380.9±7.0	18.8
Haddock meal 1	474.5± 9.3	513.1±12.1	493.8±5.9	7.8
Haddock meal 2	500.3±11.5	616.3±11.2	558.3±7.0	3.3
Haddock meal 3	518.4±10.7	587.5± 9.9	553.0±6.5	5.5
Haddock meal 4	504.2±10.9	602.9±10.2	553.6±6.6	4.4
Haddock meal 5	497.0±10.0	585.7± 7.6	541.4±5.9	4.4
Haddock meal 6	530.1± 7.6	654.5± 7.7	592.3±4.7	0
Haddock meal 7	543.7± 7.6	663.1± 8.8	603.4±4.8	0
Haddock meal 8	528.2± 8.8	573.2±16.8	550.7±9.5	7.8
Haddock meal 9	562.1± 7.2	666.0± 7.9	614.1±5.3	0
Cod meal 10	416.7± 9.4	472.4±14.9	444.6±6.1	20.1
Viscera meal 11 [1]	445.5±11.2	504.4±10.2	475.0±6.8	0

[1] Average of 3 experiments.

EXPERIMENTAL RESULTS

Table 3 shows the mean male and female weights with their probable errors, and the mean weight for the total population, on each ration. The average weight of the males and females was obtained by the formula

$$\left(M_s = \frac{A+B}{2} \pm \frac{\sqrt{a^2+b^2}}{2}\right).$$

A leg paralysis, as previously described by Norris and his associates (*6*, *7*) and by Bethke, Record, and Kennard (*1*), developed in chicks on some of the rations. The growth of chicks on the fishmeal rations was significantly greater than that of chicks on the meat-scraps ration. With the exception of viscera meal, the best growth was obtained on those rations on which the chicks showed no incidence of leg paralysis.

The rate of growth on the different supplements, with the exception of viscera meal, was in general very closely related to the percentage of leg paralysis. The chicks fed meat scraps and cod meal showed the highest incidence of leg disorder and also the poorest growth; whereas those fed meals which caused little or no leg paralysis showed significantly better growth. This suggested that the difference in growth was in part due to some factor or factors other than protein. Accordingly, in order to use growth as a criterion of the nutritive value of the proteins in the different products, it was necessary to supply those factors which would prevent the leg paralysis and also exert an effect on growth. The second series of experiments was planned to supply those factors.

Previously published (*1*) and unpublished work at the Ohio station had shown that milk products, yeast, and liver were effective in preventing the leg paralysis. These investigations also showed that it was possible to extract the protective factors from dried fat-free pork liver with cold 20-percent alcohol and eliminate a large portion of the nitrogenous products in the dried liver. This principle was used in preparing an extract from liver, on a semi-commercial scale,[4] to supply the leg-paralysis-preventing factors in the first two experiments of this series. The extract was fed at a level equivalent to 2 percent of dried liver in the total ration, which, according to previous experiments, had been found to give complete protection. This amount of extract furnished 0.25 percent of protein (N×6.25) in the ration. In the third trial, 5 percent of dried whey, containing 11.2 percent of protein, was used in place of the liver extract, because sufficient quantities of the latter were not available to complete the experiment. This amount of dried whey had been found effective in preventing leg paralysis in earlier work. The results of the experiments in which the liver extract or dried whey were used were identical. The data of the three trials are presented in table 4. The method of calculation was the same as in the previous experiments.

TABLE 4.—*Effect of different fishmeals when supplemented with liver extract or dried whey (vitamin G complex) on growth of chicks* [1]

[Average of 3 experiments]

Supplement	Average weight of chicks at 8 weeks		
	Females	Males	Males and females
	Grams	*Grams*	*Grams*
Meat scraps	445.5± 7.1	515.1± 9.5	458.0±4.6
Haddock meal 1	542.9± 7.8	605.0± 8.8	574.0±4.9
Haddock meal 2	558.7± 6.9	634.8±10.4	596.8±4.5
Haddock meal 3	555.6± 9.5	628.8± 7.7	592.2±5.6
Haddock meal 4	566.4± 6.6	647.5±10.5	607.0±6.2
Haddock meal 5	548.3± 6.6	651.4±10.1	599.9±4.3
Haddock meal 6	552.5± 7.5	642.4± 8.4	597.5±5.6
Haddock meal 7	542.0± 8.4	665.0± 9.4	603.5±6.3
Haddock meal 8	549.6±11.9	687.1± 9.5	618.4±7.0
Haddock meal 9	561.6± 8.0	667.1± 9.6	614.4±6.2
Cod meal 10	525.2±11.1	637.1± 8.8	581.2±7.1

[1] There was no leg paralysis on these rations.

[4] The writers are indebted to Dr. David Klein, of the Wilson Laboratories, Chicago, Ill., for the liver extract.

The addition of liver extract, or dried whey, to the fishmeal or meat-scraps rations prevented leg paralysis and tended to equalize the nutritive value of the different meals. The data show that the proteins of the fishmeals were superior to the proteins of the meat scraps, since, in every instance, the substitution of fishmeal for meat scraps resulted in significantly better growth. The difference in the protein value of the various fishmeals was not so great as was originally postulated. The results, however, indicate that high drying temperatures lower the protein value of the meals. In all cases the meals which were dried under vacuum gave somewhat better growth than did the flame-dried, or steam-dried products, with the exception of cod meal. The difference in growth of chicks on flame-dried and vacuum-dried meals, prepared from the whole waste, was significant in 2 out of 4 cases, and approached significance in the other 2 cases. The difference in growth of chicks on steam-dried and vacuum-dried products was not so pronounced. No outstanding difference in protein value was noted between the whole waste meals prepared by the wet-rendering and dry-rendering processes. The meal prepared from the waste of the edible portion (no. 8, backbones) gave the best growth and proved significantly better than any of the other meals, irrespective of the kind of waste or method of preparation used in their manufacture.

TABLE 5.—*Comparative efficiency of the different rations*

Supplement	Feed required per gram gain in weight—		Supplement	Feed required per gram gain in weight—	
	Without added vitamin G complex	With added vitamin G complex		Without added vitamin G complex	With added vitamin G complex
	Grams	*Grams*		*Grams*	*Grams*
Meat scraps	3.28	3.04	Haddock meal 6	2.54	2.60
Haddock meal 1	2.69	2.68	Haddock meal 7	2.52	2.56
Haddock meal 2	2.60	2.61	Haddock meal 8	2.52	2.50
Haddock meal 3	2.55	2.55	Haddock meal 9	2.55	2.59
Haddock meal 4	2.58	2.56	Cod meal 10	2.68	2.64
Haddock meal 5	2.60	2.62	Viscera meal 11	3.01	

The efficiency with which the different rations in the two series of experiments were utilized is shown in table 5. There was no difference in the utilization of the cod-meal and haddock-meal rations, but these proved more efficient than the rations supplemented with viscera meal or meat scraps.

Mortality was not a factor in any of the experiments in the first series. The few fatalities that occurred were not related to any particular supplement. In one of the three experiments in the second series, considerable mortality resulted from pullorum infection, but it was not connected with the ration. In the other two experiments of this series, mortality was negligible.

TABLE 6.—*Effect of the addition of liver extract or dried whey (vitamin G complex) on the incidence of leg paralysis and on the growth of chicks*

Supplement	With vitamin G complex added		Without added vitamin G complex		Difference in weights due to added vitamin G complex
	Average male and female weights	Leg paralysis	Average male and female weights	Leg paralysis	
	Grams	*Percent*	*Grams*	*Percent*	*Grams*
Meat scraps	458.0±4.6	0	380.9±7.0	18.8	77.1± 8.4
Haddock meal 1	574.0±4.9	0	493.8±5.9	7.8	80.2± 7.7
Haddock meal 2	596.8±4.5	0	558.3±7.0	3.3	38.5± 8.3
Haddock meal 3	592.2±5.6	0	553.0±6.5	5.5	39.2± 8.6
Haddock meal 4	607.0±6.2	0	553.6±6.6	4.4	53.4± 9.1
Haddock meal 5	599.9±4.3	0	541.4±5.9	4.4	58.5± 7.3
Haddock meal 6	597.5±5.6	0	592.3±4.7	0	5.2± 7.3
Haddock meal 7	603.5±6.3	0	603.4±4.8	0	0.1± 7.9
Haddock meal 8	618.4±7.0	0	550.7±9.5	7.8	67.7± 11.8
Haddock meal 9	614.4±6.2	0	614.1±5.3	0	0.3± 8.2
Cod meal 10	581.2±7.1	0	444.6±6.1	20.1	136.6± 9.4

DISCUSSION

For ease in comparing the results of the two series of experiments, the data are summarized in table 6. It is very evident that some of the fishmeals and the meat-scraps rations were deficient in certain factors which affected growth and caused the occurrence of a leg paralysis. Those meals (nos. 6, 7, and 9) that gave the best growth and no incidence of leg paralysis when unsupplemented were not improved by the addition of liver extract or dried whey. This fact, the writers believe, affords definite proof that the difference in growth obtained on the products in the first series was not entirely due to protein but in large measure to a partial deficiency in the vitamin G complex. The remarkably close correlation between the difference in growth on the supplemented and the unsupplemented meals and the percentage of leg paralysis gives further support to the hypothesis that growth and leg paralysis are closely associated with a deficiency of the vitamin G complex.

The results of the two series also emphasize the importance of adequately controlling the vitamin G complex content of the ration, if the correct conclusions regarding the protein value or the total nutritive effect of the product under investigation are to be reached. In fact, the data suggest that many of the supplementary protein studies of the past may have been complicated by the vitamin G complex and that the increased growth or better performance obtained was, in reality, primarily a vitamin effect and not due to protein per se.

It is worthy of note that all of the unsupplemented rations containing the meals (nos. 1, 2, 3, 4, and 10) that had been cooked and centrifuged prior to drying caused a variable incidence of leg paralysis. This condition was prevented and the rate of growth was increased by the addition of the vitamin G complex in the form of a liver extract or dried whey. From this it is apparent that the vitamin factors were partially destroyed or removed by the cooking and pressing operations. That heat treatment is destructive of the vitamin G complex is shown by the results obtained with meal 1 as compared with those

obtained with meals 2, 3, and 4, and with meal 5 as compared with meals 6 and 7.

The data also show that the meal made from the heads and tails of haddock waste (no. 9) contains a larger percentage of the vitamin G factors than the meal (no. 8) prepared from the waste of the edible portion of haddock. The latter meal, however, appeared to contain proteins which were superior to the product made from the heads and tails of the waste.

SUMMARY AND CONCLUSIONS

The comparative nutritive values of fishmeals prepared under experimentally controlled conditions have been studied in growth experiments with chicks. The experiments show that the cooking and centrifuging of the fish waste prior to drying removes some of the vitamin G complex and that high drying temperatures also exert a deleterious effect on these factors.

The proteins of vacuum-dried haddock meals were found to be superior to the proteins of flame-dried haddock meal for promoting growth. The difference in growth response of chicks fed the steam-dried and those fed the vacuum-dried products was not significant.

No significant difference in protein value, as measured by growth, was observed between the meals prepared by the wet-rendering (cooking and centrifuging) and dry-rendering (direct drying) processes. Meal prepared from the waste of the edible portion of the haddock fillet industry was superior in protein value to a product prepared from the heads and tails, or the entire waste, under similar conditions. However, the meal prepared from the heads and tails contained more of the vitamin G factors than the product prepared from the waste of the edible portion (backbones).

The fishmeal proteins were significantly better than the meat-scrap proteins for promoting growth in chicks. The proteins of cod meal and haddock meal were equally efficient for growth.

These experiments emphasize the importance of adequately controlling all vitamin factors in protein studies in which growth is used as an index.

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EFFECT OF METHOD OF MANUFACTURE ON THE NUTRITIVE VALUE OF FISHMEALS AS DETERMINED BY NITROGEN-BALANCE STUDIES WITH RATS [1]

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INTRODUCTION

In the first report of this series [2] it was shown that, as measured by growth experiments with chicks, the protein of vacuum-dried haddock meal was superior to that of a flame-dried haddock meal and that no significant difference in protein value existed between haddock meals prepared under similar drying conditions by the wet-rendering (cooking and centrifuging) and dry-rendering (drying directly) processes. Likewise, no great difference in protein value was found between steam-dried and vacuum-dried haddock meals prepared from the whole waste. On the other hand, the protein of a meal prepared from the waste of the edible portion (backbones) of the haddock fillet industry was superior to that of a meal similarly prepared from the heads and tails, or the entire waste. These differences between certain meals may be accounted for either on the basis of a difference in the digestibility of the proteins or of a lowered utilization of the absorbed nitrogen. However, the growth experiments with chicks measured only the total protein value of the respective meals and gave no information regarding the nitrogen partition. Accordingly, it was deemed desirable to extend the initial studies by the use of methods which would give more specific information regarding the difference in protein efficiency. The present paper reports the results of the nitrogen-balance studies with rats. The method described by Mitchell [3] was used.

MATERIALS USED

The meals were from the same supply as those used in the chick experiments. A description of the meals and the method of preparing them are given in the preceding paper [4] and need not be repeated here.

The soluble and insoluble fractions from haddock meal 6, fed in two trials, were prepared by adding water to the dry meal and boiling for 15 minutes and then pressing while hot. The residue was suspended in boiling water and pressed immediately. The water extracts were combined and evaporated on dextrinized cornstarch before a fan at room temperature and finally dried in an electric oven for 12 hours at 75° C. The residue was also dried at 75° for 12 hours.

[1] Received for publication Apr. 10, 1934; issued November, 1934.

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[4] RECORD, P. R.; BETHKE, R. M.; and WILDER, O. H. M. See footnote 2.

EXPERIMENTAL PROCEDURE

Young male rats of comparable history were used throughout the several trials. They were first placed upon the low-nitrogen ration, then on the fishmeal rations, and, finally, again on the low-nitrogen ration in accordance with the procedure described by Mitchell.[5] The experimental periods were 10 days or more in length. The collection of excreta was made during the last 7 days of each period. Five separate groups of rats, or a total of 34, were used.

The low-nitrogen ration consisted of 84 percent dextrinized cornstarch, 10 percent Crisco, 4 percent salt mixture (Osborne and Mendel) and 2 percent cod-liver oil. The fishmeals or fractions thereof were fed at such levels that their protein formed 10 percent of the ration. They were incorporated in the low-nitrogen ration by replacing all of the salt mixture and part of the dextrin, except in the case of the water-soluble-fraction ration, in which 4 percent of the salt mixture was included. Vitamin B was supplied daily and separately as an alcoholic extract from rice polish. The amount fed daily was equivalent to 1.5 g of rice polish and furnished 0.7 mg of nitrogen daily. A cold 20-percent alcoholic extract of defatted dried pork liver served as a source of vitamin G. This was also fed daily and separately in quantities equivalent to 500 mg of dried liver. This amount of liver extract supplied approximately 2.5 mg of nitrogen daily. These amounts of vitamin supplements furnished ample quantities of the respective factors. The rations and all excreta were analyzed for nitrogen by the Kjeldahl method.

[5] MITCHELL, H. H. See footnote 3.

TABLE 1.—*Digestibility and biological value of haddock meals 1, 2, and 3 when used as supplements to the rations of rats*

Rat no.	Body weight		Average daily food intake	Average daily N intake	Average daily fecal N	Estimated daily metabolic N	Average daily food N in the feces	Average daily absorbed N	Average daily urinary N	Estimated daily endogenous N	Average daily food N in the urine	Average daily food N utilized	Digestion coefficient (apparent)	Digestion coefficient (corrected)	Biological value of meal
	Initial	Final													
	Grams	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*	*Percent*	*Percent*
PERIOD 1. LOW-NITROGEN RATION (0.094 PERCENT N)															
7	90	85	6.04		17.9	[1] 2.96			18.3	[2] 21.0					
8	91	85	6.37		18.3	[1] 2.87			15.8	[2] 17.8					
9	89	80	5.89		18.4	[1] 3.12			17.2	[2] 20.5					
10	88	84	6.36		18.1	[1] 2.84			16.5	[2] 18.7					
11	100	92	7.22		22.1	[1] 3.06			21.2	[2] 22.2					
12	92	84	5.09		18.1	[1] 3.55			25.6	[2] 29.1					
PERIOD 2. HADDOCK MEAL NO. 2 (1.652 PERCENT N)															
7	100	115	9.10	153.2	38.1	28.6	9.5	143.7	38.1	23.2	14.9	128.8	75	93	89
8	93	110	7.45	125.9	32.5	22.6	9.9	116.0	31.8	21.3	10.5	105.5	74	92	90
9	95	116	8.91	150.0	39.0	30.1	8.9	141.1	35.6	23.8	11.8	129.3	74	94	91
10	103	116	8.30	139.9	35.8	25.3	10.5	129.4	34.7	21.1	13.6	115.8	74	92	89
11	115	128	9.50	159.8	39.1	29.0	10.1	149.7	35.4	25.8	9.6	140.1	75	93	93
12	98	115	8.13	137.1	34.7	29.4	5.3	131.8	39.0	30.3	8.7	123.1	75	96	93
Average													74±0.1	93±0.4	91±0.5
PERIOD 3. HADDOCK MEAL NO. 1 (1.683 PERCENT N)															
7	124	136	9.97	170.3	55.8	33.1	22.7	147.6	49.7	29.2	20.5	127.1	67	86	86
8	118	134	9.27	158.6	51.4	34.3	17.1	141.5	51.0	29.5	21.5	120.0	67	89	85
9	127	138	10.00	170.8	59.6	36.4	23.2	147.6	49.4	32.3	17.1	130.5	65	86	88
10	122	137	9.98	170.5	57.2	32.5	24.7	145.8	49.5	26.8	22.7	123.1	66	85	84
11	142	155	10.00	170.8	54.9	30.5	24.4	146.4	55.3	31.1	24.2	122.2	68	85	83
12	127	145	9.95	170.0	58.3	36.7	21.6	148.4	57.3	39.0	18.3	130.1	66	87	88
Average													66±0.3	86±0.4	86±0.6

[1] Per gram of food.
[2] Per 100 grams live weight.

TABLE 1.—*Digestibility and biological value of haddock meals 1, 2, and 3 when used as supplements to the rations of rats*—Continued

PERIOD 4. HADDOCK MEAL NO. 3 (1.645 PERCENT N)

Rat no.	Body weight		Average daily food intake	Average daily N intake	Average daily fecal N	Estimated daily metabolic N	Average daily food N in the feces	Average daily absorbed N	Average daily urinary N	Estimated daily endogenous N	Average daily food N in the urine	Average daily food N utilized	Digestion coefficient (apparent)	Digestion coefficient (corrected)	Biological value of meal
	Initial	Final													
	Grams	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*	*Percent*	*Percent*
7	144	155	10.00	167.3	40.1	35.0	5.1	162.2	56.4	34.5	21.9	140.3	76	96	86
8	147	161	10.00	167.3	44.4	33.4	11.0	156.3	48.6	40.5	8.1	148.2	73	93	94
9	153	168	10.00	167.3	43.5	39.0	4.5	162.8	53.4	42.4	11.0	151.8	74	97	93
10	151	164	10.00	167.3	44.3	34.7	9.6	157.7	52.8	34.0	18.8	138.9	73	94	88
11	165	178	10.00	167.3	32.6	30.4	2.2	165.1	52.5	34.9	17.6	147.5	80	98	89
12	158	168	9.31	156.0	37.8	35.0	2.8	153.2	54.6	45.9	8.7	144.5	76	98	94
Average													75±0.7	96±0.6	91±0.9

PERIOD 5. LOW-NITROGEN RATION (0.094 PERCENT N)

7	144	132	6.35		23.4	[1] 3.68			32.7	[2] 23.7					
8	148	129	5.43		19.0	[1] 3.50			40.8	[2] 29.2					
9	152	136	4.59		19.1	[1] 4.16			40.7	[2] 28.2					
10	148	138	6.42		23.7	[1] 3.68			31.9	[2] 22.5					
11	169	158	9.90		30.1	[1] 3.04			32.5	[2] 19.8					
12	142	133	4.09		15.7	[1] 3.84			38.5	[2] 27.7					

[1] Per gram of food.
[2] Per 100 grams live weight.

RESULTS OF EXPERIMENTS

The data obtained with the first series of animals, which were fed haddock meals 1, 2, and 3, are presented in full in table 1 in order that the reader may have a complete picture of the calculations, etc., involved. To conserve space, the detailed data for the other experimental rations are omitted and only the averages of the digestibility and biological values, with their respective probable errors, are shown (table 2). The coefficients of digestibility are calculated on a corrected and an uncorrected basis. In the former case the nitrogen of metabolic origin is taken into account, while in the latter case (apparent digestibility) all of the fecal nitrogen is considered as undigested nitrogen.

TABLE 2.—*Digestibility and biological values of fishmeal proteins as affected by method of processing and drying when used as supplements to the rations of rats*

Meal no.	Kind of fish waste	Part of waste	Process [1]	Vacuum	Source of heat in dryer	Rats	Digestion coefficient (apparent)	Digestion coefficient (corrected)	Biological value
						No.	*Percent*	*Percent*	*Percent*
1	Haddock	Whole	Wet	No	Direct flame	6	66±0.3	86±0.4	86±0.6
2	do	do	do	No	Steam	6	74± .1	93± .4	91± .5
3	do	do	do	Yes	do	6	75± .7	96± .6	91± .9
4	do	do	do	Yes	Hot water	6	79± .3	96± .2	91± .9
5	do	do	Dry	No	Steam	10	77± .8	93± .2	84± .9
6	do	do	do	Yes	do	14	79± .4	96± .3	87± .8
7	do	do	do	Yes	Hot water	10	78± .4	95± .5	86±1.1
8	do	Backbones	do	Yes	do	10	80± .3	97± .4	89±1.1
9	do	Heads and tails.	do	Yes	do	14	76± .3	95± .5	83± .8
10	Cod	Whole	Wet	No	Steam	8	73± .3	90± .4	85± .9
	Water-soluble fraction from haddock meal 6.					8	75±1.2	93±1.3	34±2.3
	Water-insoluble fraction from haddock meal 6.					8	73± .5	91± .8	97± .6

[1] Process refers to whether the material was cooked and centrifuged (wet) prior to drying or dried directly (dry).

It is clearly shown by the data in table 2 that the digestibility was most markedly affected by flame-drying. All the meals or fractions thereof were significantly more digestible than the flame-dried product (haddock 1). The differences between the other meals were not so great, and in many instances not significant. The difference in apparent digestibility between the steam and vacuum-dried dry-rendered (meals 5 and 6) and wet-rendered meals (meals 2 and 3) were 3 ± 0.8 and 4 ± 0.8, respectively; whereas the corrected digestibility coefficients for the same meals did not show any difference. Likewise, there was no significant difference between the wet- and dry-rendered meals dried under vacuum with hot water (meals 4 and 7). In the case of the wet-rendered meals (meals 2, 3, and 4) dried without and under vacuum, there was a minimum difference of 3 ± 0.4 in corrected digestibility between the meals in favor of the vacuum-dried products. On the other hand, the difference in the apparent digestibility between the same meals was significant only in the case of the vacuum-hot-water-dried product (meal 4) over the steam-dried product (meal 2). The difference in digestibility between the vacuum- and steam-dried dry-rendered whole-waste haddock meals was not so great as in the case of the wet-rendered products.

Only in the case of the corrected digestibility coefficients were the differences between the vacuum-dried meals (6 and 7) significantly greater than the steam-dried (meal 5) product (3 ± 0.4 and 2 ± 0.5, respectively). The apparent as well as the corrected digestibility coefficients of the meal prepared from the waste of the edible portion (meal 8) were significantly greater to the extent of 2 ± 0.5 and 2 ± 0.6 than the respective coefficients for the proteins of the whole-waste product (meal 7); and 4 ± 0.4 and 2 ± 0.6 greater than the protein of the meal made from the heads and tails (meal 9). The digestibility data also show that the proteins of dry-rendered or wet-rendered whole-waste haddock meal was more digestible than similarly dried whole-waste wet-rendered cod meal.

The water-soluble nitrogenous constituents of the vacuum-dried, dry-rendered meal were practically as well digested as the nitrogen compounds of the other meals and significantly better than those of the flame-dried meal (meal 1). On the other hand, both the apparent and corrected digestibility coefficients of the water-insoluble protein (residue) were significantly lower than those of the other meals with the exception of haddock meals 1 and 2, cod meal 10, and the water-soluble fraction. The protein of the residue was, however, significantly better digested than the protein of the flame-dried product (meal 1).

The data also show that the biological value of the meals was adversely affected by high drying temperatures, as previously reported by Maynard and Tunison,[6] and by the method of processing the waste prior to drying. A minimum difference of 5 ± 1.1 in favor of vacuum or steam-drying over flame drying is shown in the case of the wet-rendered products. No significant difference was found in the utilization of the absorbed nitrogen between the steam-dried and vacuum-dried meals prepared by either the dry- or wet-rendered process. Likewise, the biological value of the flame-dried haddock-meal protein (meal 1) was comparable to the protein of the steam- or vacuum-dried dry-rendered products (meals 5, 6, and 7). The steam- and vacuum-dried wet processed meals, however, were significantly superior in biological value to the similarly dried dry-processed products. Meals 2, 3, and 4 (wet-rendered) showed a difference of 7 ± 1.0, 4 ± 1.2, and 5 ± 1.4 over the dry-rendered meals 5, 6, and 7, respectively.

A comparison of the meals prepared from different parts of haddock waste, under similar conditions, reveals a respective difference of 3 ± 1.5 and 6 ± 1.4 in biological value in favor of the product made from the waste of the edible portion (backbones) over that made from the whole waste (meal 7) and from the heads and tails (meal 9), indicating a significant difference in biological value only in the latter case.

Of particular interest is the remarkably low biological value of the water-soluble nitrogen fraction and the high value of the water-insoluble residue. The absorbed nitrogen utilized in case of the insoluble residue was significantly greater than for any of the meals regardless of source or treatment. All of the rats on the water-soluble-fraction ration consumed on an average only 3.96 to 4.99 g of feed daily and either lost or remained stationary in weight during the

[6] MAYNARD, L. A., and TUNISON, A. V. INFLUENCE OF DRYING TEMPERATURE UPON DIGESTIBILITY AND BIOLOGICAL VALUE OF FISH PROTEINS. Indus. and Engin. Chem. 24: 1168–1171. 1932.

experimental period. The animals were in negative nitrogen balance, which makes the observed biological figures of doubtful value. Nevertheless, the writers are of the opinion that the great difference noted in nitrogen utilization between the water-soluble and water-insoluble fractions affords a direct explanation of the higher biological values obtained with the wet-rendered meals, since only from 10 to 15 percent of the total nitrogen in the wet-processed products was removed by treating the different meals with water at 100° C. for 5 minutes, and from 20 to 25 percent of the total nitrogen was removed from the dry-processed whole waste products by similar treatment. Likewise, 23.4 percent of the nitrogen in the meal made from the heads and tails of the haddock waste (meal 9) was water-soluble, in contrast with 13.3 percent for the total nitrogen in the meal prepared from the waste of the edible portion (meal 8). This difference in water-soluble nitrogen constituents between meals 8 and 9 accounts, in part at least, for the superior biological value of the protein in the former meal (meal 8).

The results of the nitrogen-balance experiments with rats are of particular interest when compared with the results of the growth studies with chicks.[7] In the case of the chick experiments, no significant difference was observed between the protein value of the whole waste haddock meals that were dried under similar conditions and prepared either by the wet- or dry-rendering process when the rations were adequately supplemented with the vitamin G complex (liver extract or dried whey); yet the biological values of the wet-processed meals, as determined with rats, were significantly higher than those of the dry-processed. The latter meals, however, appeared to posess a slightly greater apparent digestibility, which fact no doubt tended to offset, in part, the higher biological value of the wet-rendered meals, so that the total protein value of the two differently processed meals as measured in the chick experiment was approximately equal. It should also be pointed out that in all probability the level at which the protein was fed in the chick experiments was too high to measure accurately the small differences in total protein value between certain meals.

Another point of difference between the results of the chick and rat experiments existed in the case of the similarly prepared cod meal and haddock meal (meal 2). In the chick experiments no significant difference was observed in protein value between these two meals when the rations included the liver extract or dried whey (vitamin G complex); whereas in the case of the nitrogen partition experiments the haddock meal proved significantly superior in digestibility and biological value.

Aside from these exceptions, the results of the chick and rat experiments were in close agreement.

SUMMARY

High drying temperatures, as used in flame drying, significantly lowered the digestibility and biological value of the protein in haddock meal. No difference was noted in the utilization of the absorbed nitrogen between steam-dried and vacuum-dried meals when prepared by either the wet- or dry-rendering process. The protein of the vac-

[7] Record, P. R., Bethke, R. M., and Wilder, O. H. M. See footnote 2.

uum-dried meals, however, appeared to be slightly more digestible than that of the steam-dried haddock products.

The method of processing the fish waste prior to drying significantly affected the biological value of the protein in the meal. The absorbed nitrogen from the wet-rendered meals was utilized more efficiently than that from the dry-rendered products.

The higher biological value of the wet- over the dry-processed whole-waste haddock meals was, in all probability, due to the lower percentage of water-soluble nitrogen constituents which were shown to be of exceptionally low biological value. From 20 to 25 percent of the total nitrogen in the dry-processed meals was soluble in hot water in contrast to 10 to 15 percent in the wet-rendered meals.

The protein of the haddock meal prepared from the waste of the edible portion (backbones) was significantly more digestible than that of meals similarly prepared either from the whole waste or the head and tails. The backbone meal (meal 8) also proved significantly superior to the meal prepared from the heads and tails as regards the utilization of the absorbed nitrogen. In the case of the meals prepared from the whole waste and the waste of the edible portion, the difference in biologidal value was not biometrically significant.

The protein of the wet-rendered steam-dried haddock meal was more digestible and possessed significantly greater biological value than that of the meal similarly prepared from the whole waste of the cod.

DIAMETER DISTRIBUTIONS FOR OLD-FIELD LOBLOLLY PINE STANDS IN MARYLAND[1]

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EXPLANATION OF THE PRESENT STUDY

Although European foresters have made use of diameter frequency distributions, or stand tables, for the past 50 years, it is only within the last decade that American forest mensurationists have focused their attention upon them. Baker (*1*)[3] first used this method of analysis in North America in 1923, when he showed that diameter distributions in certain timber stands closely approximate the "normal frequency distribution." Since that time more and more interest in diameter distributions has been manifested until, at present, stand tables[4] are considered an essential part of timber yield tables.

Graphic methods of stand-table construction were developed by Bruce and Reineke (*2, 3, 10*) between 1926 and 1929. These methods apply one curve type to all stands regardless of age or average stand diameter. Mathematical methods of computing frequencies, developed by Charlier (*4*), and used by Ilvessalo (*6*) in Finland and Schumacher (*12, 13*) and Meyer (*7*) in the United States, fit separate curves to each average stand diameter class. They recognize four particular stand characteristics having distinct trends and following the same general law in most of the species studied.

Meyer (*7*) made a number of comparisons between the mathematical and graphic methods and found that the latter removed the above-mentioned trends and introduced a possibility of serious error.

One of the species studied by Bruce was loblolly pine (*Pinus taeda* L.) for which the Forest Service (*14*) made a general stand table in connection with a southern pine yield study. Though this was a comprehensive study, including loblolly stands from the whole southern pine region, it did not include a study of the distribution characteristics of individual stands. Recently an opportunity for more intensive study was presented during the analysis of permanent sample-plot records. The results of this study, a combination of graphical and mathematical methods, is presented here. The object of this combined method is to utilize both the rigidity and impersonality of the mathematical processes for determining and fitting the type curve, and the flexibility of graphical presentation for rapid solution in the application to practical problems.

In 1906 the Forest Service established 58 permanent sample plots in old-field loblolly pine stands on private lands in Worcester County, Md. These have been remeasured at approximately 5-year intervals

[1] Received for publication June 13, 1934; issued November, 1934.
[2] Maintained at Philadelphia, Pa., in cooperation with the University of Pennsylvania.
[3] Reference is made by number (italic) to Literature Cited, p. 743.
[4] Stand tables show numbers of trees by diameter classes at different ages or different average stand diameters.

up to the present time in cooperation with the Maryland State Department of Forestry. However, because of insufficient measurements, less than normal stocking (below eight-tenths crown cover as estimated in the field), thinnings, and abandonment, only 34 of these plots could be used for the study of diameter distributions in normal stands, yielding 111 individual plot measurements (table 1).

TABLE 1.—*Successive basic plot measurements in old-field loblolly pine*[1]

Plot no.	Plot area	April 1906		June 1910		June 1915		July 1920		February 1926		February 1930		Plot measurements
		Trees	Mean d.b.h.	Trees	Mean d.b.h.	Trees	Mean d.b.h.	Trees	Mean d.b.h.	Trees	Mean d.b.h.	Trees	Mean d.b.h.	
	Acre	*No.*	*In.*	*No.*	*In.*	*No.*	*In.*	*No.*	*In.*	*No.*	*In.*	*No.*	*In.*	*No.*
14	0.10	191	[2] 3.51											1
15	.10	172	3.93	119	4.58	93	5.34	67	[3] 6.06					4
16	.25	360	4.30	237	5.46	186	6.30	141	[3] 7.11					4
17	.25	335	[2] 4.35											1
18	.20	254	4.55	180	5.39	146	6.15	117	[3] 6.98					4
19	.20	263	[2] 4.49											1
20	.25	280	[2] 4.62											1
21	.25	256	4.82	163	6.23	132	7.03	117	7.70	105	8.41	88	9.12	6
22	.10	587	.96	177	3.00	107	4.95	81	5.83	62	6.92	51	7.67	6
26	.50	(4)	(4)	(4)	(4)	449	3.94	246	6.42	214	7.40	184	8.32	4
27	.50	712	[3] 3.55											1
28	.50	738	3.51	669	4.18	539	4.97	368	6.00	300	6.81	265	7.38	6
29	.30	328	[2] 4.88											1
30	.40	422	5.02	284	6.11	212	7.22	145	[3] 8.32					4
31	.25	767	2.11	211	4.24	178	5.22	146	[3] 6.26					4
32	.25	366	[2] 4.16											1
33	.25	427	[3] 3.42											1
34	.20	424	3.05	198	4.90	145	6.19	110	7.50	92	8.59	79	9.40	6
35	.25	323	[2] 4.20											1
36	.25	285	4.87	207	6.02	167	7.13	124	8.14	106	[3] 8.77			5
38	.50	4,582	.61	2,399	1.98	1,118	3.29	555	4.56	361	5.93	288	6.63	6
39	.25	2,345	.55	1,410	1.73	735	2.92	331	4.17	200	[3] 5.42			5
41	.20	127	5.91	105	6.69	81	7.44							3
42	.20	132	[2] 5.99											1
43	.10	28	10.13	28	10.91	27	[3] 11.37							3
52	.20	389	2.87	235	4.15	194	[3] 4.91							3
54	.20	452	[2] 2.74											1
56	.10	213	2.80	107	4.65	89	5.34	69	[3] 6.25					4
57	.25	93	8.68	77	9.36	65	10.02	60	[3] 10.75					4
60	.10	220	2.99	116	4.26	74	[3] 5.03							3
61	.125	266	3.01	137	4.21	108	[3] 4.90							3
62	.125	274	3.04	130	4.28	93	[3] 4.98							3
63	.10	335	1.83	(4)	(4)	133	4.84	80	6.25	63	7.28	52	8.14	5
66	.25	(4)	(4)	804	2.08	563	3.00	261	4.68	185	5.82	155	6.52	5
Total number of plots														111

[1] Intervals between measurements represent 5 growing seasons with the exception of the last, which is 4. "Mean d.b.h."=mean diameter at breast height.
[2] Thinned after this measurement.
[3] Abandoned after this measurement.
[4] Insufficient measurements.

On each plot, every tree having a diameter breast high[5] of 2.5 inches and more was measured in tenths of inches and recorded separately; those below 2.5 inches were tallied as 0.5 to 1.4 inches and 1.5 to 2.4 inches. Prior to 1926 the diameter measurements were obtained with calipers, the average of two measurements taken at right angles being used. The 1926 and 1930 diameter breast high measurements were obtained with a diameter tape. In 1926 Reineke (*9*) made a study of the difference between tape and caliper measurements on these plots and found practically none.

[5] At 4.5 feet above average ground level.

CHARACTERISTICS OF DIAMETER DISTRIBUTIONS

Diameter distributions of the trees in stands of timber can be represented by a number of mathematical expressions, namely:

Number of trees, which refers to the total number of trees on any given plot (table 1).

Mean diameter, which is the size of the average tree, obtained by totaling the diameters of all the trees on the plot and dividing by the number of trees (table 1).

Standard deviation, which is a measure of dispersion of the diameters around the mean diameter—that is, whether they are grouped close to the mean or scattered far from it; the ratio of the standard deviation to its mean, expressed as a percentage, is the coefficient of variation.

Coefficient of asymmetry, which is a measure of the skewness of the distribution to one side or the other of the mean; that is, whether the class having the greatest number of trees is above or below the mean.

Coefficient of excess, which is a measure of the pointedness or flatness of the distribution curve with corresponding variations in the tails.

These characteristics were computed for each plot, using the method outlined by Meyer (7). The values thus obtained were averaged[6] by 1-inch mean stand diameter classes, each plot being considered as a separate stand, regardless of size or number of trees. The averages were plotted on graph paper and weighted curves drawn as shown in figure 1.

A comparison of the trends of these characteristics with those obtained by Schumacher and Meyer show them to be quite similar. Standard deviation increases with diameter increase, though not at a constant rate. The initial rapid rate of increase is probably due to the fact that all trees below 4.5 feet in height were excluded, thus lowering the standard deviation. Ratio of deviation to mean decreases with diameter increase, with some tendency to increase again in the higher diameters. Coefficient of asymmetry is negative and has a linear trend, but the correlation with mean stand diameter is not statistically significant. This means that the bunching of trees is in the classes a little below the mean, but remains in the same relative place regardless of stand diameter. Coefficient of excess is also linear, but is not statistically significant from zero.

AVERAGE DISTRIBUTION FOR ALL PLOTS

Since the excess is zero and the skewness practically constant for all stands regardless of mean diameter, there are but two characteristics free to vary, namely, number of trees and standard deviation. In order to combine all of the plots to get one average diameter distribution, the mean of each plot was equated to zero and the number of trees, as a percentage of the total, was determined by one-half standard deviation classes.[7] This was accomplished by making a graph of cumulative frequency of number of trees, in percentages,

[6] Average standard deviation was obtained from the average of the squared standard deviations.
[7] This method was used by Schumacher to combine plots within 2-inch mean diameter classes.

over the diameter breast high for each plot, and reading from these curves the value of the upper limit of each one-half standard deviation class. Frequency values were then obtained from these by sub-

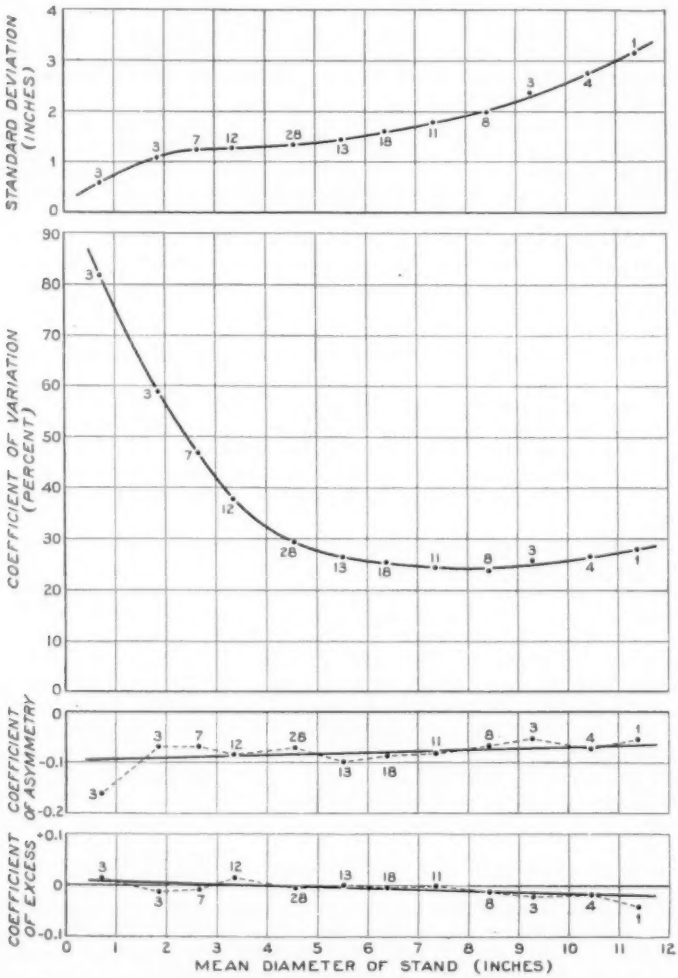

FIGURE 1.—Relation of diameter distribution characteristics to mean diameter of stand.

traction. Table 2 and figure 2 illustrate the method for plot 21, as measured in 1906.

Table 3 illustrates the method of obtaining the frequencies in standard units. The frequencies for all of the plots were then added together and averaged by standard units to obtain one average frequency distribution for the 111 plots (column 2, table 4).

TABLE 2.—*Frequency of plot 21 (1906) used in figure 2*

Diameter breast high class [1]	Trees in each class	Cumulative tally of trees [2]		Diameter breast-high class [1]	Trees in each class	Cumulative tally of trees [2]	
Inches	*Number*	*Number*	*Percent*	*Inches*	*Number*	*Number*	*Percent*
0.5–1.4	10	10	3.9	5.0–5.9	54	187	73.0
1.5–2.4	10	20	7.8	6.0–6.9	48	235	91.7
2.5–2.9	9	29	11.3	7.0–7.9	15	250	97.6
3.0–3.9	46	75	29.3	8.0–8.9	6	256	100.0
4.0–4.9	58	133	51.9				

[1] Group inconsistencies are due to manner in which field measurements were taken.
[2] Percentile values are those used in figure 2.

TABLE 3.—*Method of converting frequency from regular to standard units*

Upper limit of each 0.5 standard deviation class	Diameter breast high [1]	Cumulative tally of trees [2]	Trees in each class [3]	Midpoint of standard deviation class [4]	Upper limit of each 0.5 standard deviation class	Diameter breast high [1]	Cumulative tally of trees [2]	Trees in each class [3]	Midpoint of standard deviation class [4]
	Inches	*Percent*	*Percent*			*Inches*	*Percent*	*Percent*	
−2.75	0.260	1	1	−3.0	+0.25	5.240	58	18	+0.0
−2.25	1.090	2	1	−2.5	+.75	6.070	75	17	+.5
−1.75	1.920	5	3	−2.0	+1.25	6.900	91	16	+1.0
−1.25	2.750	10	5	−1.5	+1.75	7.730	96	5	+1.5
−.75	3.580	23	13	−1.0	+2.25	8.560	99	3	+2.0
−.25	4.410	40	17	−.5	+2.75	9.390	100	1	+2.5
Mean 0	4.825								

[1] Values computed from the mean diameter breast high using 0.25 and 0.5 standard deviation values. (Mean diameter breast high = 4.825 inches, standard deviation = 1.66 inches, 0.5 standard deviation = 0.83 inch, 0.25 standard deviation = 0.415 inch.)
[2] Values read from fig. 2, using column 2 diameters.
[3] Computed from column 3 by successive subtractions.
[4] The point at which column 4 values apply.

SELECTION OF TYPE CURVE

It is quite possible to fit a smooth frequency curve to these points and regard it as the average frequency of the 111 plots. However, it was desired to go further than this and get the average frequency curve for the whole universe of timber stands which might occur in this species, type, and region, so that it would be applicable to any stand in the region.

Therefore, a mathematical type curve to fit the data was looked for, in the realization that the type that would most closely fit the frequency distribution would most likely be the correct one, provided the sample is representative of the whole. Accordingly, five different curves were fitted to the average frequency distribution, the computed values of which are given in table 4.

Charlier's type A; type B, 2-term; and type B, 4-term curves were fitted first. The work involved in fitting these curves is not very great because functional tables are available. The type B, 4-term curve fitted best of these three but required a greater amount of work. In order to get the very exact fit, since that might be expected in the application to an average frequency, Pearson's method (*5*, *8*) was tried. The constants β_1 and β_2 show the distribution to be Pearson's type 1. Computation was a laborious job but gave fine results. One more curve was tested, Pearson's type 3, because of the simplicity in fitting by the use of Salvosa's (*11*) tables of the type 3 function. It was found to fit about as well as Charlier's type A.

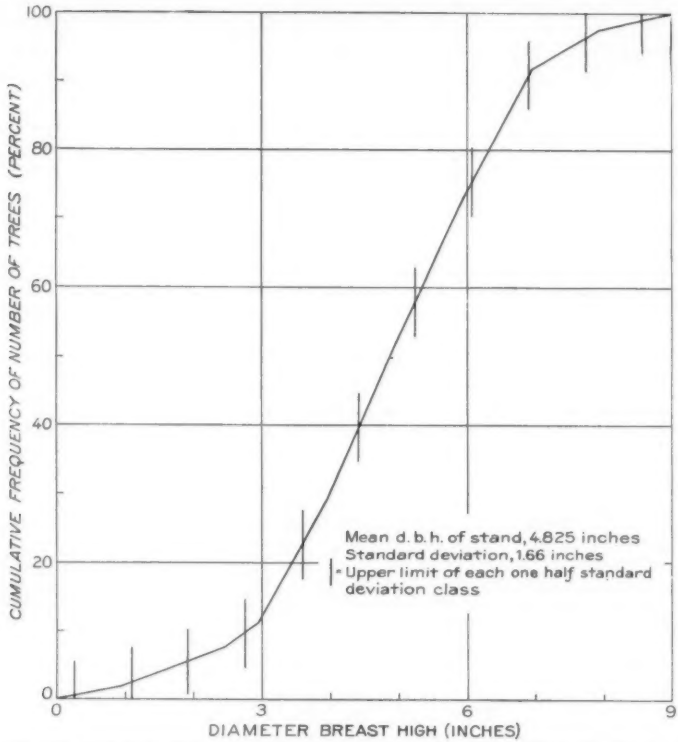

FIGURE 2.—Cumulative frequency of number of trees for sample plot 21 (1906), showing method of converting from diameter classes to one-half standard deviation classes.

TABLE 4.—*Comparison of computed frequencies*

Mid-point of standard deviation class	Average frequency distribution for all plots	Computed frequencies				
		Pearson's type I	Charlier's type B, 4-term	Charlier's type A	Pearson's type 3	Charlier's type B, 2-term
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
−3.0	0.01	0.00	−0.16	−0.16	0.00	0.03
−2.5	.21	.03	+.08	+.22	.18	.61
−2.0	1.58	1.87	2.47	2.24	1.80	2.92
−1.5	7.35	7.86	7.91	7.22	6.94	7.60
−1.0	15.84	14.94	14.50	14.37	14.48	13.38
−.5	19.32	19.06	18.82	19.76	19.78	17.66
Mean 0	18.08	18.68	18.80	19.80	19.84	18.43
+.5	15.17	15.09	15.36	15.32	15.72	15.80
+1.0	10.23	10.42	10.52	9.94	10.33	11.35
+1.5	6.45	6.26	6.19	5.89	5.84	6.89
+2.0	3.34	3.30	3.15	3.22	2.92	3.51
+2.5	1.53	1.53	1.47	1.52	1.31	1.48
+3.0	.59	.62	.58	.57	.54	.46
+3.5	.22	.22	.24	.16	.20	.07
+4.0	.07	.06	.05	.03	.08	−.06
+4.5	.00	.02	.01	.00	.02	−.06
+5.0	.01	.00	.02	.00	.01	−.03

In figure 3 are presented graphical comparisons of the fit of these five type curves, placed in order of ease of fitting, Pearson's type 3

requiring the least and Pearson's type 1 curve requiring the greatest amount of computational work. All of the curves fit the data quite closely. A sensitive test, a mathematical measure of the relative

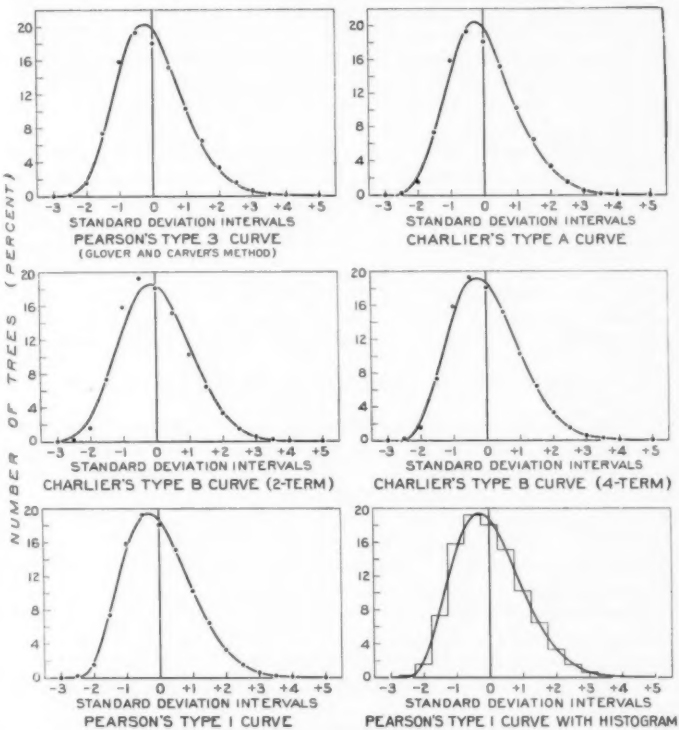
NUMBER OF TREES (PERCENT)
STANDARD DEVIATION INTERVALS
PEARSON'S TYPE 3 CURVE
(GLOVER AND CARVER'S METHOD)
CHARLIER'S TYPE A CURVE
CHARLIER'S TYPE B CURVE (2-TERM)
CHARLIER'S TYPE B CURVE (4-TERM)
PEARSON'S TYPE I CURVE
PEARSON'S TYPE I CURVE WITH HISTOGRAM

FIGURE 3.—Comparison of fit of several type curves to the average frequency distribution of three diameters, obtained from 111 permanent sample plot measurements.

goodness of fit of these curves, is available in Pearson's (*8*) χ^2 test, the results of which were as follows:

Pearson type 1	0. 1260
Charlier type B (4-term)	. 3838
Charlier type A	. 4969
Pearson type 3	. 5107
Charlier type B (2-term)	1. 8337

Low values of χ^2 mean good agreement; high values, poor. Pearson's type 1 curve, as expected, had the smallest χ^2 value.[8] However, Charlier's type B, 4-term curve fits very nearly as well, while Charlier's type A and Pearson's type 3 fit reasonably well. Charlier's type B, 2-term curve fits much less closely than the others.

Since these are average points, a type curve of exceptionally good fit must be selected if it is to be regarded as the best estimate of the timber type which these plots represent. Pearson's type 1 curve

[8] Ordinarily χ^2 tests are not applied to average frequencies or to distributions expressed as percentages because of high internal correlations. In this case they are used for comparative purposes only.

though not a perfect fit has a very low χ^2 value and was accordingly selected as the best type curve available.

To find out how well this type curve fits stands of different average diameters, the average distribution points for each 1-inch mean stand diameter class were plotted about the curve as shown in figure 4. It is noticed that the curve fits the points fairly well regardless of stand diameter. The points for classes containing but few plots naturally are fitted much less accurately. That the 0- to 0.9-inch class is very erratic may be due to the omission of trees under 4.5 feet in height.

To test the fit of the type curve to an individual plot, graphic comparisons with the consecutive measurements of plot 21, chosen at random, were made as shown in figure 5. It is not expected that any one plot, which represents but a small area of standing timber, will fit the average type curve exceedingly well. However, the actual frequency hovers around the average, being skewed above in 1906 and gradually swinging back until in 1920 it is below the average, then back to a more positive skewness in 1930. At all times the smooth curve encloses an area which is very close to that defined by the actual data.

CONSTRUCTION OF STAND ALINEMENT CHART

Since the tests substantiated the use of the one computed type curve for all stands, the next step was to put this curve into an easily applied form. A cumulative frequency curve (fig. 6, *A*) was constructed by using cumulative values computed from column 3 in table 4. This curve was anamorphosed into a straight line, thereby modifying the vertical scale. This straight line connects the limits −2.73 standard units and +8.91 standard units, the computed limits of the type curve, between which, on an average, lie all of the trees in any stand. Since this type distribution is to be used for all stand diameters, it was but necessary to compute the limits for different stand diameters, plot them, and draw straight lines between them. Table 5 gives the computations of the limits and figure 6, *B*,[9] presents the plotted values.

TABLE 5.—*Computations of diameter limits*

Mean diameter of stand	Standard deviation [1]	Standard deviation multiplied by—		Diameter limits	
		−2.73	+8.91	Lower [2]	Upper [3]
Inches	*Inches*			*Inches*	*Inches*
0.5	0.45	−1.2285	4.0095	[4] −0.7285	4.5095
1.0	.74	−2.0202	6.5934	−1.0202	7.5934
1.5	.96	−2.6208	8.5536	−1.1208	10.0536
2.0	1.13	−3.0849	10.0683	−1.0849	12.0683
3.0	1.26	−3.4398	11.2266	−.4398	14.2266
4.0	1.30	−3.5490	11.5830	+.4510	15.5830
5.0	1.38	−3.7674	12.2958	1.2326	17.2958
6.0	1.53	−4.1769	13.6323	1.8231	19.6323
7.0	1.72	−4.6956	15.3252	2.3044	22.3252
8.0	1.92	−5.2416	17.1072	2.7584	25.1072
9.0	2.18	−5.9514	19.4238	3.0486	28.4238
10.0	2.54	−6.9342	22.6314	3.0658	32.6314
11.0	3.00	−8.1900	26.7300	2.8100	37.7300
12.0	3.49	−9.5277	31.0959	2.4723	43.0959

[1] Values read from fig. 1.
[2] Column 1 plus column 3.
[3] Column 1 plus column 4.
[4] Negative diameter values are caused by presence of trees under 4.5 feet in height, the point of diameter measurement.

[9] Irregularities in the lower part of this chart are due to abnormalities resulting from exclusion of all trees below diameter breast high (4.5 feet in height).

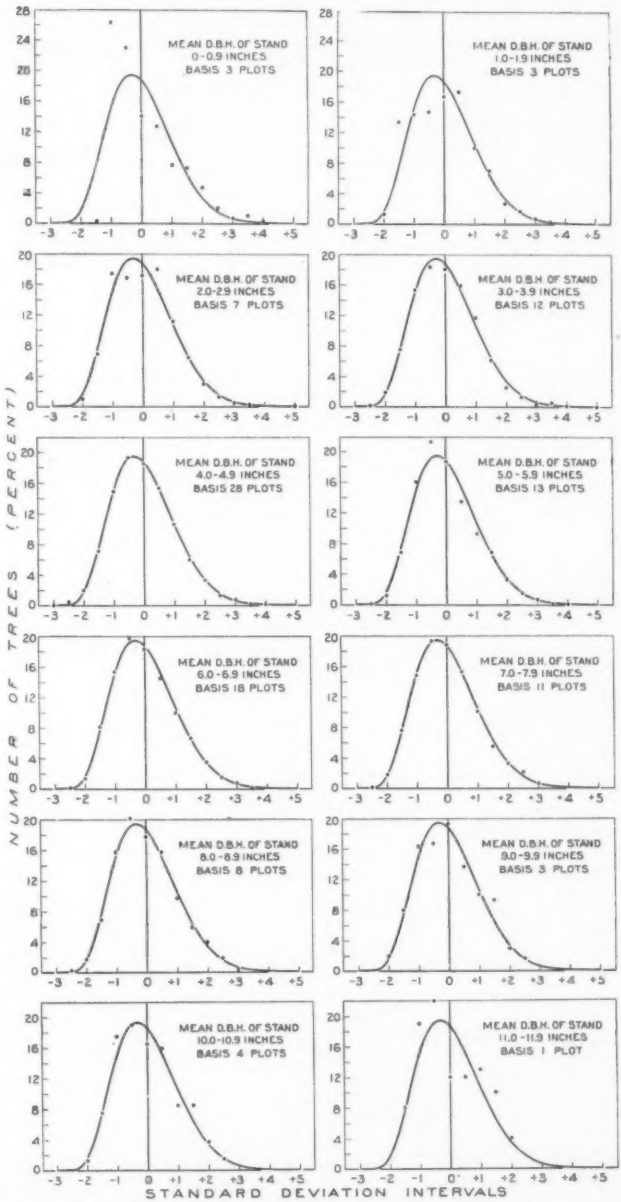

FIGURE 4.—Comparison of fit of Pearson's type 1 curve for all plots to the average frequency for each 1-inch mean diameter breast high class.

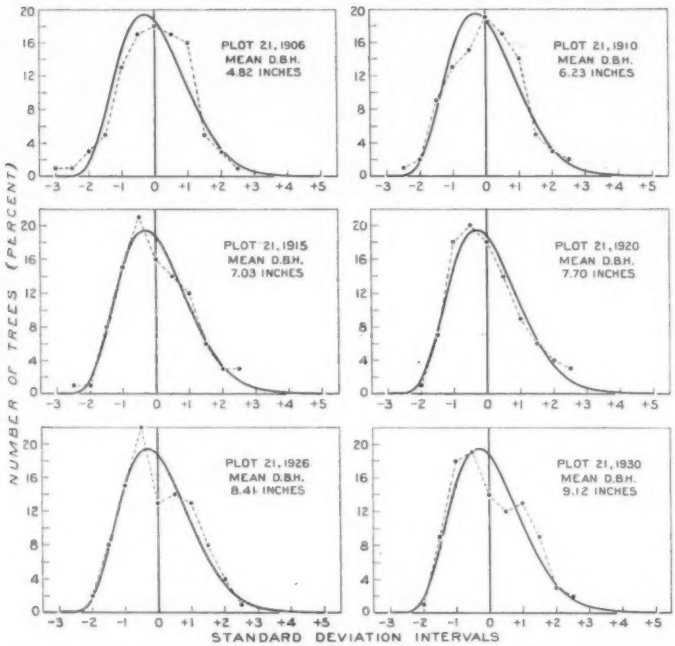

FIGURE 5.—Comparison of fit of Pearson's type 1 curve for all plots to the periodic measurements of an individual plot (plot 21).

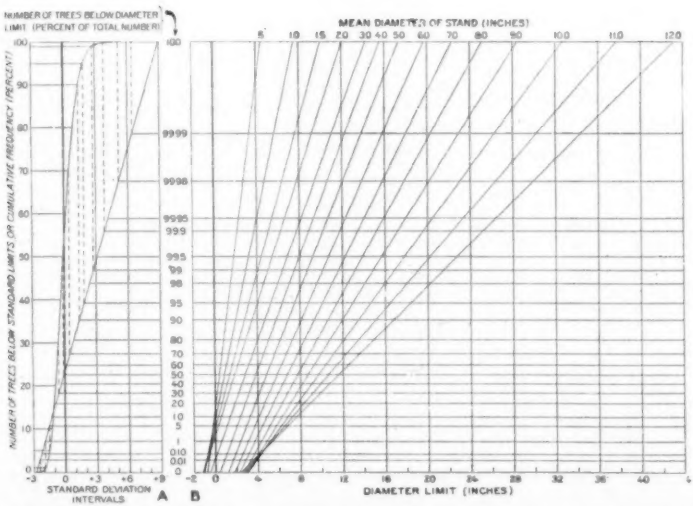
NUMBER OF TREES BELOW DIAMETER LIMIT (PERCENT OF TOTAL NUMBER)
MEAN DIAMETER OF STAND (INCHES)
NUMBER OF TREES BELOW STANDARD LIMITS OR CUMULATIVE FREQUENCY (PERCENT)
STANDARD DEVIATION INTERVALS
A
B
DIAMETER LIMIT (INCHES)

FIGURE 6.—Stand probability chart. *A*, cumulative frequency curve and method of anamorphosing into a straight line; *B*, cumulative frequency lines for successive mean stand diameter classes.

With values read from figure 6, *B*, the stand table alinement chart in figure 7 was constructed, using the method described by Bruce and Reineke (*3*). From this chart stand tables can be computed in the usual way.

DISCUSSION

The results of this intensive study indicate some improvement over the stand table presented in 1929 (*14*). In the latter, skewness

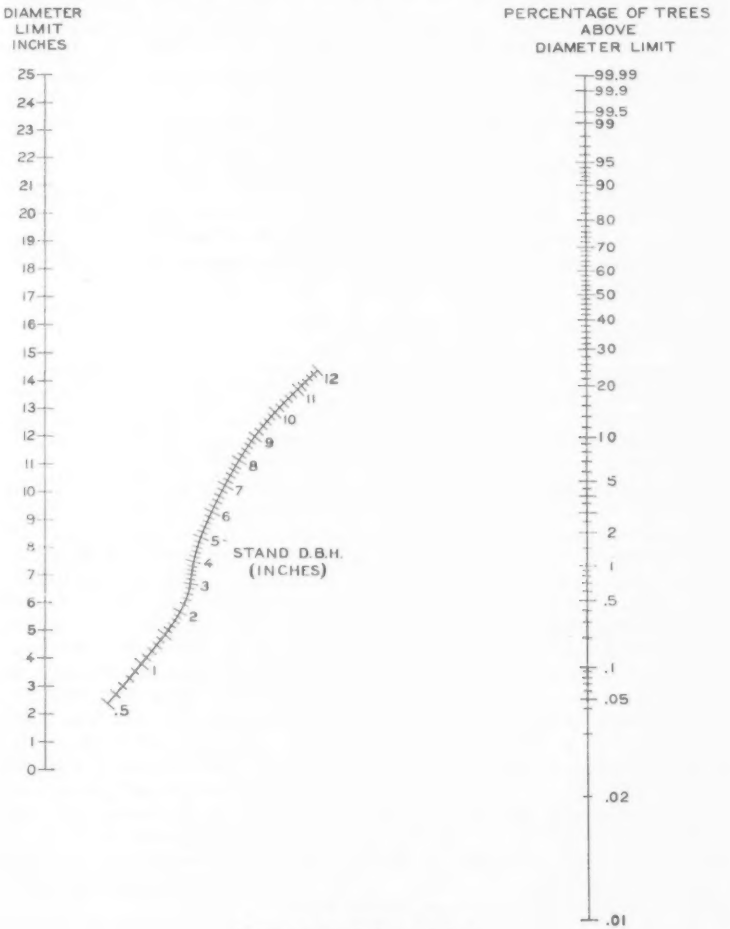

FIGURE 7.—Stand alinement chart.

decreases from positive to negative values with increasing stand diameter, a trend distinctly out of line with the results of both Schumacher (*12, 13*) and Meyer (*7*) in their work with a number of other species. In no case did these investigators find decreasing trends. The results of the present study agree with those of Schu-

macher and Meyer. The 1929 (*14*) table shows, however, a stabilizing of skewness in stands above 12 inches average diameter. This stabilized value is exactly the same as the average value computed in the present study. The indications are, therefore, that for stands averaging 12 inches in diameter or less, the present study embodies more nearly the true degree of skewness.

Differences of from 2 to 5 percent as between the two studies are also shown in the extreme diameters of the distributions. These are rather important in the larger diameter classes. For instance, in a stand having an average diameter of 12 inches, the 1929 stand table shows only 9 percent of the trees to be 16 inches in diameter breast high and larger, while figure 7 of this paper shows 14 percent. A 12-inch stand has approximately 200 trees per acre, so the 5-percent difference indicated would be 10 trees. A difference of 10 trees per acre above 16 inches in diameter is quite important to the forest owner, because these trees contain not only the higher quality material but also in this case more than 10 percent of the total basal area of the stand. Since both graphic comparisons, figures 4 and 5, show good agreement in the right-hand tails of the frequency curves, which represent the larger diameters, the present frequencies are believed to be more nearly correct.

SUMMARY

Mathematical methods of diameter distribution, such as have been used by European foresters for a number of years, have been combined in this study with the graphical methods developed by American foresters during the last decade, to effect a method at once more accurate for curve fitting and more flexible in application.

The diameters of all trees from 111 plot records formed the basic material. Of five computed mathematical expressions that definitely represent diameter size distribution, only two, number of trees and standard deviation, varied significantly with varying stand diameter. With these, combination of the distributions of all plots was feasible and was accomplished by using standard units of diameter and expressing number of trees in percentages. To the average diameter distribution thus obtained, 5 type curves were fitted: (1) Pearson's type 3; (2) Charlier's type A; (3) Charlier's type B, 2-term; (4) Charlier's type B, 4-term; and (5) Pearson's type 1. Pearson's type 1 curve fitted the average points most perfectly and was therefore selected as the best curve available. Graphic tests show that this one curve fit the average frequencies of various stand diameter classes very well. Consecutive measurements over a period of years on an arbitrarily selected plot were also found to fit reasonably well.

In order to facilitate application of this average curve to stands of various sizes, a stand-probability chart was constructed by using original diameter units. This was then simplified by transformation into the customary alinement-chart form.

Comparisons of the distributions obtained from this chart with those published by the Forest Service in 1929 for loblolly pine indicate improved accuracy both in skewness for stands 12 inches in diameter or less, and in the important upper classes of the diameter distributions.

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A DISEASE OF THE ENGLISH MORELLO CHERRY CAUSED BY BACTERIUM PRUNI [1]

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INTRODUCTION

During the period 1931 to 1933 the writer received from Missouri leaves and fruit of English Morello cherry (*Prunus cerasus* L.) affected with a bacterial disease. He has collected similarly diseased English Morello leaves at Fayetteville, Ark., and has received cankered English Morello limbs and twigs from Iowa. Pure cultures of a yellow bacterium resembling *Bacterium pruni* E. F. Smith [3] were readily secured from the diseased tissues, and it was suspected that *Bact. pruni* was the cause of the disease.

Although *Bacterium pruni* has been known since 1902 as the cause of a serious disease of plums, peaches, nectarines, and apricots, there have been only isolated reports [4] of its occurrence on cultivated cherries in the United States. These reports have been merely statements that spots, considered to be the result of leaf invasion by *Bact. pruni*, were observed on the leaves of various sweet and sour cherries, but so far as the writer can ascertain no data have been published that would establish definitely that these spots were caused by *Bact. pruni* or that *Bact. pruni* could produce a disease on cultivated cherries under natural conditions. It is true that for several years Sackett [5] believed he had isolated *Bact. pruni* from the Wragg cherry in Colorado, but he concluded finally that the pathogene was a new species, to which he gave the name *Phytomonas cerasi wraggi*, without publishing a description of the organism or stating in what respect it differed from *Bact. pruni*.

[1] Received for publication May 31, 1934; issued November 1934.

[2] Acknowledgment is made to the authorities of the College of Agriculture, University of Arkansas, for the use of the laboratories and other facilities of the Department of Plant Pathology. The writer is indebted to H. B. Johnson, Division of Fruit and Vegetable Crops and Diseases, who first observed the disease in Missouri; to M. A. Smith, of the same Division, for the specimens from Missouri; and to H. Ness, assistant State entomologist of Iowa, for specimens from that State.

[3] This organism also may be called *Pseudomonas pruni* E. F. Smith or *Phytomonas pruni* (E. F. Smith) Bergey et al., depending upon the system of classification followed.

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PATHOGENICITY AND IDENTIFICATION OF THE ORGANISM

The pathogenicity of the cultures secured in 1931 was demonstrated by fulfilling Koch's postulates in a series of inoculation experiments on English Morello cherry trees at Fayetteville, Ark. These cultures and new pathogenic isolations from additional material in 1932 and 1933 were used in cross-inoculation experiments. Typical spots were produced on peach leaves by the organism isolated from the English Morello cherry, and typical spots were produced on the leaves and fruit of the English Morello cherry with cultures of *Bacterium pruni* obtained from the peach. It was possible also in a few cases to produce the marked distortion of the English Morello fruit (a symptom discussed later) with cultures of *Bact. pruni* from the peach and with the yellow bacterium isolated from English Morello cherry by merely spraying an aqueous suspension of the organism on the young fruit. Because of the destruction of the crops by frost no peaches were available for the reciprocal inoculations on peach fruit with cultures isolated from the English Morello cherry material.

The results of these cross-inoculation experiments, together with detailed bacteriological studies which have failed to reveal any marked divergence in morphology or in physiology between the English Morello pathogene and *Bacterium pruni*, demonstrate that the organism isolated by the writer from English Morello leaves and fruit from Missouri, English Morello leaves from Arkansas, and English Morello twigs from Iowa is *Bact. pruni*. Since the morphology and physiology of *Bact. pruni* have been reviewed in detail by the writer in a recent publication,[6] they are not discussed here.

DESCRIPTION OF THE DISEASE

SPOTS ON LEAVES

The spots on the cherry leaves do not differ in appearance from those caused by *Bacterium pruni* on peach and plum leaves, except that the writer has not observed the small circular pale-green spots on the cherry leaves that are the first symptoms of infection on the other hosts. In all the specimens examined, the presence of the disease is manifested by the development of angular, purple to purplish-brown spots (fig. 1.) 1 to 2 mm long, surrounded by a light-green border or halo. Larger spots, at times roughly circular in outline, may be formed by the fusion of several small spots.

The spots produced by the bacterial organism frequently cannot be distinguished readily on the upper surface of the leaves from those caused by the cherry-leaf-spot fungus (*Coccomyces hiemalis* Hig.), especially when the spots resulting from the fungus infection are very numerous and the individual spots remain small (fig. 2, *A*) and when the number of bacterial infections on one leaf is approximately the same as the number of fungus infections on another leaf. When these conditions do not obtain or when the number of fungus infections is so large that extensive areas of the leaf are involved by the coalescing of the individual spots, the two diseases generally can be distinguished by examination of the upper surface. The difficulty in distinguishing between the two diseases disappears when the under

[6] DUNEGAN, J. C. THE BACTERIAL SPOT DISEASE OF THE PEACH AND OTHER STONE FRUITS. U.S. Dept. Agr. Tech. Bull. 273, 53 pp., illus. 1932.

surface of the leaves is examined (fig. 2, *B*). The bacterial lesions stand out as dark-brown, water-soaked spots, frequently covered by the glistening films of bacterial exudate; whereas the fungus lesions are brownish gray and frequently are covered with white masses of spores. Archer[7] in Iowa observed this similarity between *Coccomyces* leaf spots and leaf spots that he considered were caused by *Bacterium pruni*.

SPOTS ON FRUIT

The disease appears on the immature fruit in the form of circular spots, 0.5 mm or less in diameter, having a deeper green color than that of the surrounding tissues. These spots gradually enlarge and by the time they attain a diameter of 2.0 mm (fig. 3, *A*) the centers are slightly sunken and the margins are irregular and have a water-soaked appearance. This water-soaked appearance is more apparent than real, since closer examination shows that the color of the margin is not solid but has a stippled appearance due to the premature development of red color in localized areas under the epidermis. Small dried conical masses of bacteria are frequently observed extruding from the centers of these small spots (fig. 3, *B*).

FIGURE 1.—Spots resulting from the natural infection of English Morello cherry leaves by *Bacterium pruni*. ×about 1.

Practically all the diseased immature cherries examined had more than one center of infection, but as the spots enlarged there was considerable fusion of the individual spots. The lower half of the cherry (fig. 3, *C*) seems to be the favored site for infection.

The larger spots (fig. 3, *C*), 2.5 to 5.0 mm in diameter, have reddish brown centers which are very decidedly sunken and are irregular in outline, with the epidermis wrinkled and folded. The tissues are discolored inwardly to the stone. Cellular collapse follows the discoloration, and the tissues in the affected area dry into a tough membrane covering the stone.

There is some further increase in the size of the spots as the season progresses, but the distortion of the fruit (fig. 4, *A*), resulting in slab-sided, flattened, or even triangular-shaped cherries, is due to the fact that the tissues in the areas invaded by the bacteria die while the surrounding living tissues expand in size as maturity approaches.

[7] ARCHER, W. A. See footnote 4.

This marked distortion of the fruit is the most characteristic symptom of the disease on the English Morello cherry.

The appearance of the young spots on the cherry strongly resembles that of spots of similar size produced by *Bacterium pruni* on susceptible varieties of plum, but the writer has never observed spots on plums in

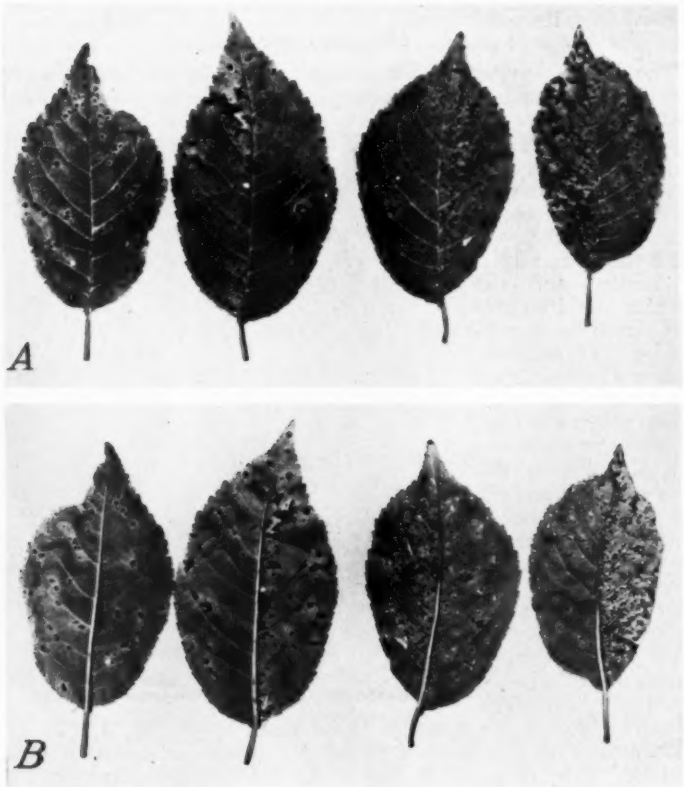

FIGURE 2.—A comparison of the symptoms produced by *Bacterium pruni* (two leaves on left) and by *Coccomyces hiemalis* (two leaves on right): *A*, Upper surface; *B*, lower surface of same leaves. × about 1. Natural infections.

which all the tissues between the epidermis and the stone were affected. Consequently, the marked distortion present on the mature cherries has not been observed on mature plums. The same statement can be made in regard to young spots on peaches, but there is even less resemblance at maturity between the lesions on the peach and those on the cherry. The roughened surface of an affected peach, resulting from the increase in diameter after infection has occurred, is analogous to the distortion produced on the cherry by the subsequent growth of the tissues surrounding the invaded areas.

LESIONS ON FRUIT STEMS

Numerous small elliptical dark-brown lesions were observed on the fruit stems, particularly those bearing infected cherries. The leaf-spot fungus, *Coccomyces hiemalis*, also was abundant on the fruit

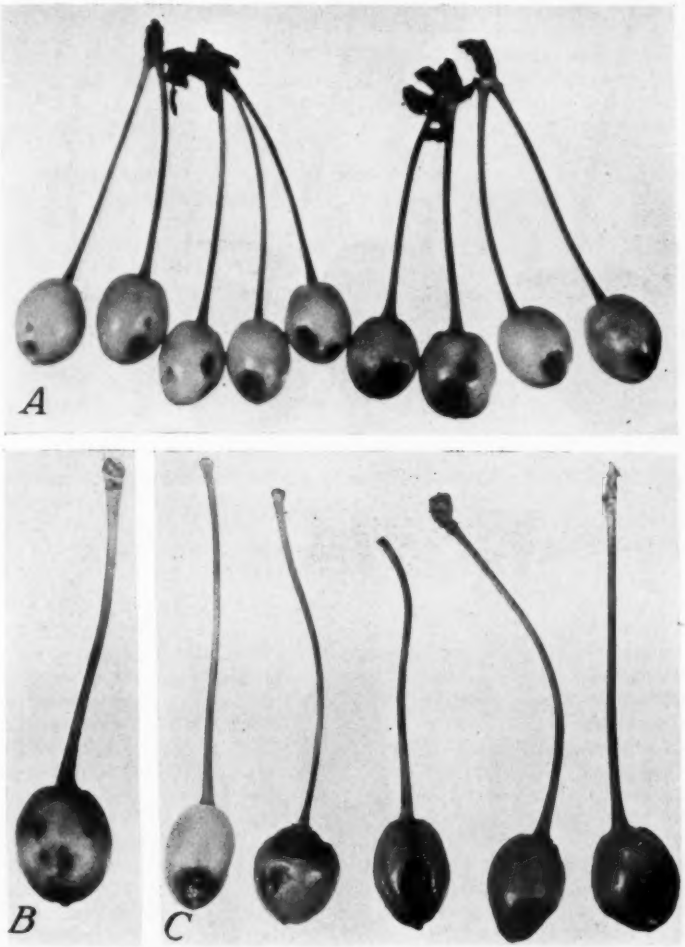

FIGURE 3.—*A*, Spots resulting from *Bacterium pruni* infections on immature English Morello cherries, × 1; *B*, masses of bacteria oozing from small spots, × 2; *C*, later stage in the development of spots on the fruit. × 2. Natural infections.

stems in the specimens examined, and the spots produced by the two organisms frequently were intermingled, making it extremely difficult to distinguish between them. Although the organism has not been isolated from lesions on the fruit stems, the existence of masses of

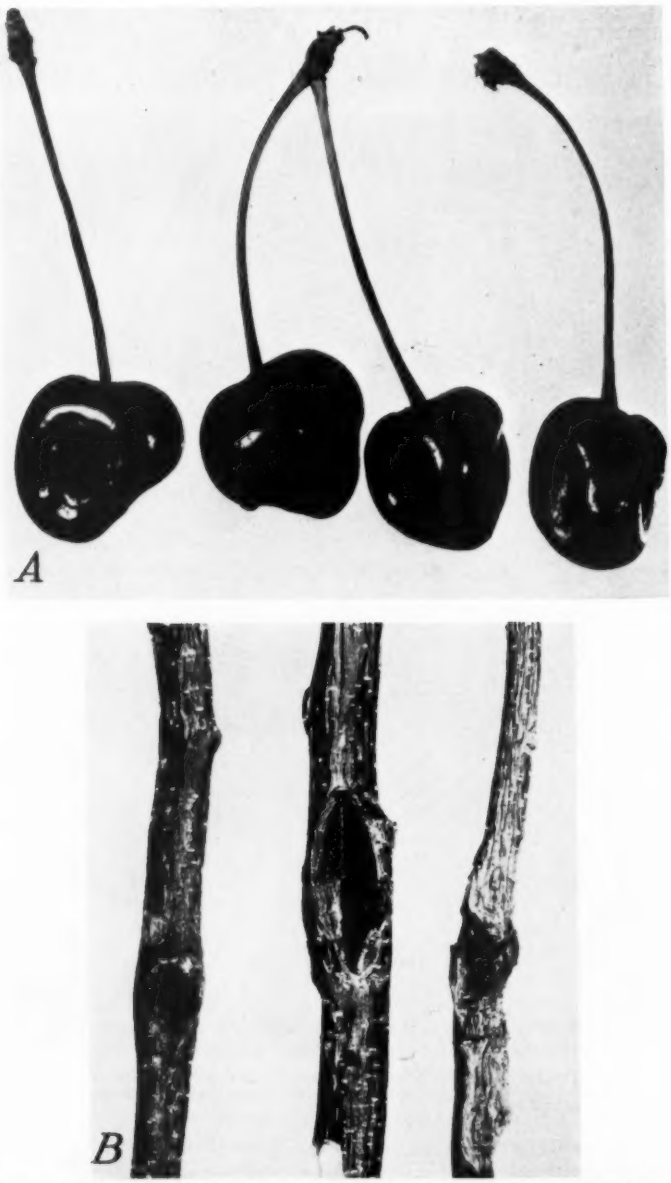

FIGURE 4.—*A*, Mature English Morello cherries showing distortion resulting from infections by *Bacterium pruni*. × 2. *B*, Overwintered cankers on English Morello cherry twigs. Pathogenic cultures of *Bact. pruni* were isolated from cankers similar to these. × about 1. Natural infection.

bacteria in cavities under the epidermis of the fruit stems was verified by a microscopic examination of sections of these stems.

CANKERS ON TWIGS

On various occasions elliptical brown cankers, 3.0 to 10.0 mm long with slightly sunken centers and water-soaked margins, were observed on the twigs of English Morello cherry material received from an orchard near Seymour, Mo. These cankers presumably were caused by *Bacterium pruni*, but all attempts to isolate the organism from them resulted in failure. *Bact. pruni* was isolated from overwintered cankers present on the cherry-twig material received from Iowa in 1933. These cankers are very similar in appearance to the cankers of the same age produced by *Bact. pruni* on the twigs of susceptible varieties of plum, as described and illustrated by Lewis [8] and others. The cherry-twig cankers (fig. 4, *B*) are roughly elliptical in outline, from 1.5 to 4.0 cm long with open centers and irregular margins produced by the development of varying amounts of wound callus. The cankers frequently coalesce, producing rough distorted regions on the twigs, 6.0 to 7.0 cm long. In the center of the cankers the wood is exposed and the blackened remnants of the cortical tissues adhere irregularly to the wood and to the wound callus developing at the sides. The twig, instead of being terete, is flattened in the vicinity of the cankers as the result of the presence of the organism in the tissues, and the fissures and cracks present on the twigs are undoubtedly the result of the tensions set up as the twigs increased in diameter. The most serious distortion occurred on the smaller twigs, where the fusion of several adjacent cankers involved the entire circumference of the twig. The cankers on the larger twigs generally do not involve the entire circumference of the twig, but their presence, nevertheless, results in a marked distortion.

DEVELOPMENT OF THE DISEASE

The development of the disease on the English Morello cherry does not differ markedly from the typical development of the bacterial spot disease on the peach and plum. The primary infections on the leaves and small cherries are derived from bacteria overwintered in the tissues of the twigs. The actual correlation of overwintered twig cankers with the primary outbreak of the disease the following spring has not been established in the orchard near Seymour, Mo., but since pure cultures of the pathogene have been isolated in the spring from the overwintered cankers received from Iowa, it is logical to suppose that the cankers have the same role on the cherry as they do on the peach and plum.

The time of the appearance of the primary infections and the subsequent development of the disease depends upon environmental factors, especially rainfall. At Seymour in 1931 and 1933 many leaves and fruits were affected early in June, and the primary infections must have occurred in May. In these 2 years rains were frequent during May and June. In 1932, however, little rain fell during May and June, the first spots were not observed on the leaves until June 15, and the disease did not become prevalent until early in July. The fruit was not affected in 1932.

[8] LEWIS, I. M. A BACTERIAL CANKER OF PLUM TWIGS. Amer. Micros. Soc. Trans. 31: 145–149, illus. 1912.

There is a copious production of bacterial exudate on the young leaf and fruit spots, and secondary infections on the leaves may occur throughout the growing season. Secondary infections also appear to be rather common on the small green cherries, but have been observed only rarely on the cherries after they begin to turn red and acquire the acid reaction of the mature fruit. The scarcity of infections after the cherries begin to develop color indicates the possibility that if the primary infections do not occur while the fruit is still small and green the fruit may escape infection. This was the situation in 1932.

The bacterial exudate from the leaf and fruit spots is also probably the main source of inoculum in the production of the spots frequently found on the fruit stems and the necrotic areas observed on the twigs at various times during the growing season.

PATHOLOGICAL HISTOLOGY

Microtome sections of fruit spots in different stages of development have been prepared to ascertain whether the distortion of the mature cherries is explainable on an anatomical basis. These sections reveal that aside from the physiological difference doubtless existing between the cells of the cherry fruit and those of the peach and plum, there are certain anatomical differences which may account, in part at least, for the unusual symptoms on the mature cherries.

In the peach and the plum the subepidermal cells of the fleshy pericarp differ in size and shape from the flesh cells. This condition extends downward 6 to 10 cell layers and constitutes the region which, for want of a better name, may be called the hypodermis. This term, however, should not lead to the supposition that there are two distinct tissue systems. All the cells are parenchymatous in nature and a gradient exists from the flattened cells just beneath the epidermis to large isodiametric cells of the flesh parenchyma. A radial section of the outer portion of an English Morello cherry reveals only a layer or two of flattened parenchyma cells between the epidermis and the large parenchyma cells of the flesh.

In the peach and the plum *Bacterium pruni* enters through the stomata and produces a distinct cavity in the hypodermis, and only rarely does the cavity involve the flesh parenchyma cells. In the English Morello cherry *Bact. pruni* enters through the stomata, but since there is practically no hypodermis the flesh parenchyma cells are affected almost immediately; and the bacteria, instead of being limited to the periphery of the fruit, may be found in the intercellular spaces of the flesh cells from the epidermis to the region of the stone. While the walls of many of the parenchyma cells collapse, others in the invaded region are affected only partially and tend to hold the fragments of collapsed walls together so that a definite cavity does not appear to be formed.

In the peach and the plum, by the time a cavity is formed or shortly thereafter, a periderm is initiated in the vicinity of the invaded region and the diseased portion is separated from the healthy tissues by a layer of wound cork.

In the English Morello cherry no evidence of a periderm forming wound cork has been observed in any of the sections. Eventually all the cells in the invaded region collapse and the cellular debris adheres to the stone as a tough membrane. In view of the total destruction

of the tissues in the invaded region it is remarkable that the organism does not involve the entire fruit. The parenchyma cells in the region between the invaded tissues and the healthy tissues, becoming meristematic, produce a compact mass of cells smaller than the normal flesh cells, which seems to prevent further invasion.

DISTRIBUTION OF THE DISEASE

No definite information is available concerning the distribution of the disease aside from the fact that it has been recognized on specimens from Missouri, Arkansas, and Iowa. Since the bacterial spot occurs on other members of the genus *Prunus* in all the fruit-growing States east of the Rocky Mountains, it is probable that it is widely distributed on the English Morello and other varieties of cultivated cherries but has not been recognized as a distinct disease. The marked similarity between the symptoms produced on the leaves by the bacterial organism and by the cherry leaf-spot fungus may be one reason for this. The distortion of the mature fruit, a symptom quite different from the general effects of *Bacterium pruni* on fruit, may be another reason.

ECONOMIC IMPORTANCE OF THE DISEASE

With little definite information available concerning the distribution of the disease, its economic importance can be estimated only from its effects on the orchard near Seymour where it has been under observation for 3 years.

It is obvious that distorted fruit such as is depicted in figure 4, *A*, is worthless for market purposes, and that the presence of the disease on the fruit year after year would result in serious loss. The observations made near Seymour indicate, however, that the fruit escapes infection in some seasons and that even in those years when the disease is present on the fruit the extent of the injury varies from tree to tree. No instance was observed in which the entire crop of an individual tree was ruined. The presence of the distorted cherries increases the cost of packing, as each cherry must be examined carefully to eliminate the misshapen fruits.

SUMMARY

The English Morello cherry is very susceptible to attacks of the cherry leaf-spot fungus, and therefore it is difficult to evaluate the injury to the foliage and to the succeeding crops of fruit resulting from the activities of the bacterial pathogene. That it causes some defoliation is certain, but the leaf-spot fungus also is capable of completely defoliating the trees; and when both diseases are present it is impossible to determine definitely how much defoliation is due to the bacterial organism alone.

In the orchard near Seymour the pathogene seemed to have little effect on the twigs, but some of the smaller twigs received from Iowa were badly distorted and weakened by the cankers. It is possible that young twigs may be killed by cankers that girdle the young developing shoots. The presence of cankers complicates the control problem because they act as the source of the overwintered inoculum that initiates the infection the following spring.

A yellow bacterium, identified as *Bacterium pruni*, has been isolated from leaves, fruit, and twigs of diseased English Morello cherry (*Prunus cerasus*).

The symptoms on the leaves, fruit, fruit stems, and twigs of the English Morello cherry are described in detail.

The pathogene presumably overwinters in the tissues of the twigs. The appearance of the primary infections is dependent upon environmental factors and may vary from season to season. Secondary infections on the leaves, fruit stems, and twigs may occur throughout the growing season. On the small green cherries secondary infections are somewhat common, but they have been observed only occasionally after the cherries turn red and begin to mature.

The pathological histology of the disease on the fruit is discussed. The bacteria are present in the tissues from the epidermis to the stone and eventually cause a complete destruction of all the cells in the invaded region. Certain anatomical differences were noted which may account for this complete destruction of the tissues as compared with the localized effect of *Bacterium pruni* on the tissues of peaches and plums.

The disease has been identified only on specimens from Missouri, Arkansas, and Iowa, but it is believed to be widely distributed. The similarity between the symptoms produced on the leaves by *Bacterium pruni* and *Coccomyces hiemalis* and the unusual symptoms on the mature fruit have probably delayed its recognition as a distinct disease.

In some seasons the disease, by producing a distortion in the shape of the mature cherries, may cause a reduction in the size of the merchantable crop. This distortion also increases the cost of packing the crop, since the misshapen cherries must be sorted out and discarded. The leaf-spot phase of the disease is responsible for a certain amount of defoliation, but it is difficult to evaluate this injury. The cankers distort and weaken the twigs and are indirectly of economic importance in the overwintering of the organism.

SOME PLANT-PARASITIC NEMAS, WITH DESCRIPTIONS OF THREE NEW SPECIES[1]

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INTRODUCTION

Investigations of the nemic fauna frequently add new information concerning the host relationship and distribution of well-known species and occasionally reveal new, undescribed forms. This report brings

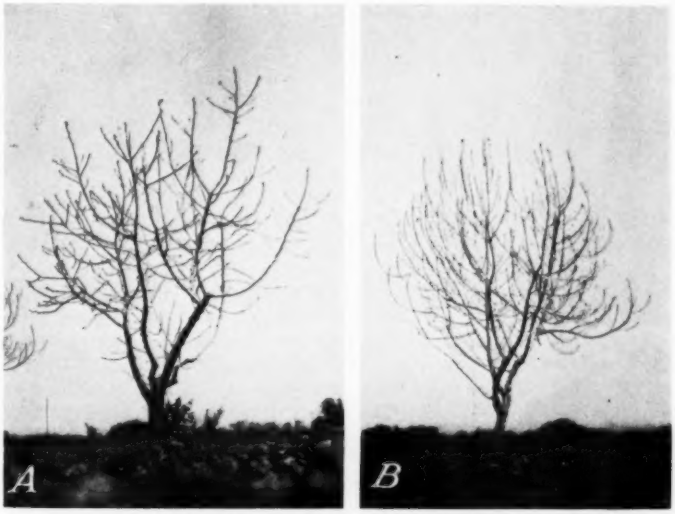

FIGURE 1.—*A*, Mission fig tree infested with *Anguillulina pratensis; B*, uninfested tree.

together the results of several minor projects dealing with plant-parasitic species.

ANGUILLULINA PRATENSIS

The known distribution of *Anguillulina pratensis* (De Man)[3] Goodey in the roots of figs (*Ficus carica* L.) is confined to California, in 2 orchards near Merced, in 1 at Modesto, and in 1 near Riverside. This nema was first observed attacking fig-tree roots by Cobb[4] in 1927, but diseased trees had been observed in about 1920.[5]

Severely infested trees are readily distinguished by their retarded growth and by a conspicuous reduction in the number and size of branches (fig. 1, *A*). Usually the taproots of such trees are dead

[1] Received for publication July 13, 1934; issued November 1934.

[2] The writer is indebted to H. N. Hansen, of the University of California, Davis, Calif., for infested material, and to C. E. Scott and D. P. Wheeler, of the California State Department of Agriculture, for data on the distribution of *Anguillulina pratensis* and for aid in visiting the orchards near Merced, Calif. The California State Department of Agriculture kindly allowed the use of photographs used in fig. 2, *E*, *F*.

[3] Synonym: *Tylenchus penetrans* Cobb.

[4] COBB, N. A. TYLENCHUS PENETRANS COBB. Jour. Parasitol. 14: 71. 1927.

[5] Oral communication by Dr. Ralph Smith, Berkeley, Calif.

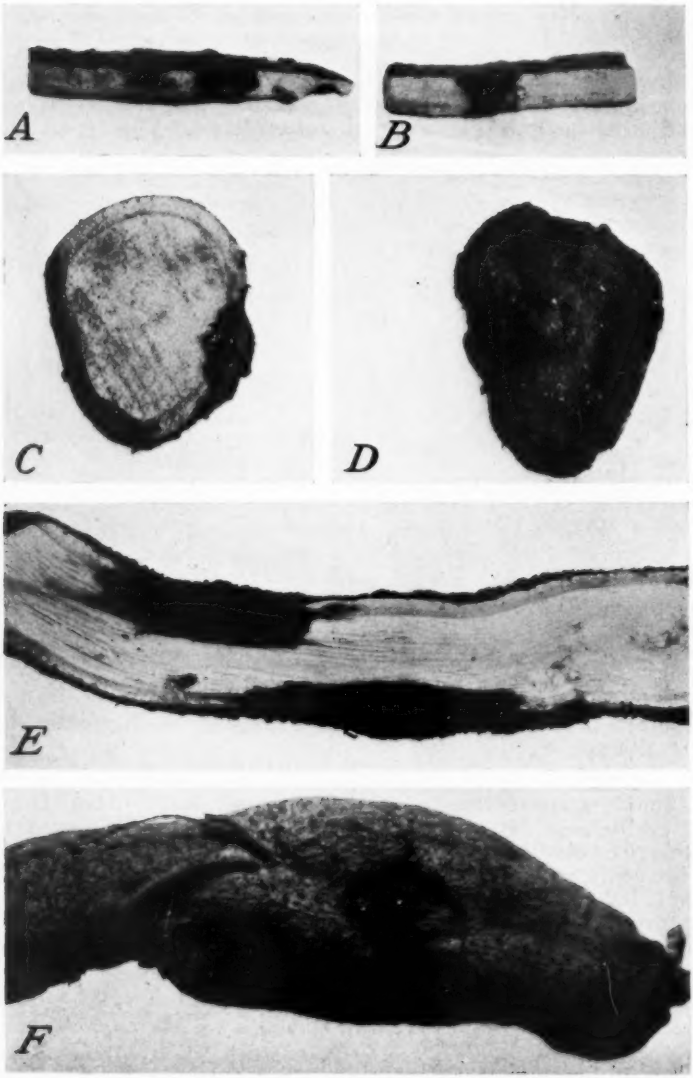

FIGURE 2.—*A* and *B*, Sections of bark, showing new lesions caused by *Anguillulina pratensis*; *C*, cross section of root, showing lesions; *D*, cross section of root entirely decayed; *E*, longitudinal section of infested root; *F*, flattened root, a condition frequently associated with infestations of *A. pratensis*.

and the lateral roots show various degrees of damage, ranging from slight injury to complete destruction. In some instances the roots are so reduced that it is possible to push the trees over.

Points of infestation appear first on the roots as small bark lesions, which gradually extend inward (fig. 2, *A* and *B*). As the nemas advance into the root the injured tissues decay (fig. 2, *C* and *E*) until finally the root is severed (fig. 2, *D*). During this process there is a tendency for the tissue to attempt to grow around and so repair the injury, with the result that the root frequently presents a flattened appearance (fig. 2, *F*).

The nemas advance into the living root and are followed by fungi and bacteria, which produce decay and complete breakdown of the tissues. Rarely is this species found remaining in the decayed portions.

The owner of an orchard near Merced reported that the infested trees were imported from Riverside. Locally grown trees in the same orchard were not infested, indicating that, in this case, nursery stock was the source of infestation.

Distribution from tree to tree is easily possible by means of irrigation water and cultural tools. Roots near the surface are frequently cut off by plows and cultivators and the fragments carried over the orchard.

In Utah, *Anguillulina pratensis* is a common inhabitant of cultivated fields in Salt Lake, Davis, Weber, and Utah Counties. Alfalfa (*Medicago sativa* L.) frequently is infested. Generally the nemas are found scattered through the roots, but occasionally small colonies form definite lesions. The damage caused appears to be negligible. The sugar beet (*Beta vulgaris* L.) is another common host. When the plants are small, especially before thinning, the nemas may cause considerable damage by boring into the young roots.

ANGUILLULINA PUSTULICOLA, N. SP.[6]

Grass bearing pustulelike lesions containing nemas of the species *Anguillulina pustulicola* was collected from the tail skid of an airplane entering the United States from Mexicali, Mexico. Similar specimens were collected from hay originating at Nogales, Mexico.[7] Unfortunately the grass could not be identified from the small fragments submitted.

[6] Erroneously reported as *Tylenchus angustus* in: FLEURY, A. C. PLANT QUARANTINE SERVICE. Calif. Dept. Agr. Monthly Bull. 19: 816. 1930.

[7] Collected by Paul Howard, horticultural commissioner, San Diego, Calif. Received through the courtesy of D. B. Milbrath, of the California State Department of Agriculture, November 1929.

The galls first appear as slight oval stem swellings which develop into elongate, pustulelike bodies averaging 2 mm in length and 0.5 mm in thickness (fig. 3, *A*). A single stem bore nodulelike galls (fig. 3, *B*). Their color ranges from light brown to black, the discoloration frequently extending 2 to 3 mm above and below the gall. A cross section shows the gall to be of spongy, honeycombed structure, the component cells of which are much larger than those of normal grass stems. The remains of 3 to 5 female nemas, together with hundreds of preadult larvae, were present in each gall. No males were found in the several galls examined. Unfortunately, the material was very

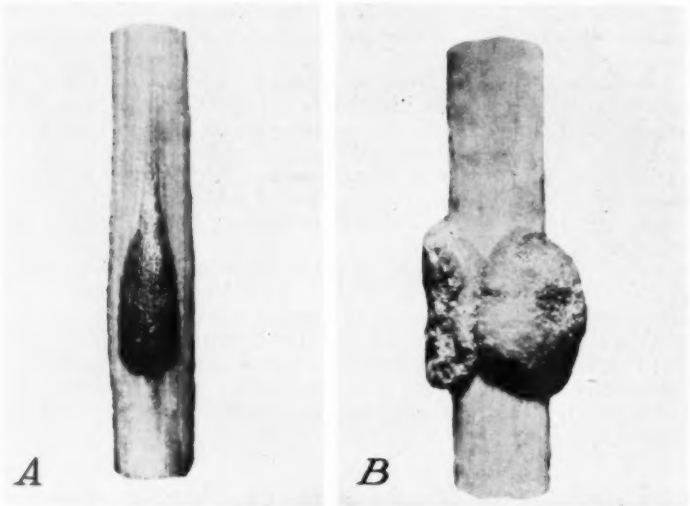

FIGURE 3.—*A*, Typical gall on grass stem, caused by *Anguillulina pustulicola*: *B*, gall of an unusual type. × 15.

dry and the specimens were in poor condition; the immature forms did not revive when moistened.

MORPHOLOGY

Female.—Length, 1.3 to 1.8 mm; width, 80μ to 95μ. Body obese, tapering anteriorly to a subhemispherical lip region continuous with neck contour. Spear 10μ to 12μ long, strongly knobbed. Vulva, near 96 percent, large, elevated. Average size of eggs, 42μ×86μ. Anus inconspicuous. Tail conoid to subacute terminus.

Quiescent stage.—Length, 0.67 to 0.74 mm; width, 16μ to 20μ. Body nearly cylindrical throughout the greater part of its length, tapering anteriorly to a rounded lip region that is not set off in any manner. Spear, 10μ long, knobbed. Genital primordium, near 60 percent. Anus usually invisible. Body tapering uniformly to subacute terminus. Male unknown.

Diagnosis.—*Anguillulina* with above measurements. Differentiated from related species by the continuous lip region and specific pathogenic effects on host. Causal agent of pustulelike galls on grass stems (fig. 4).

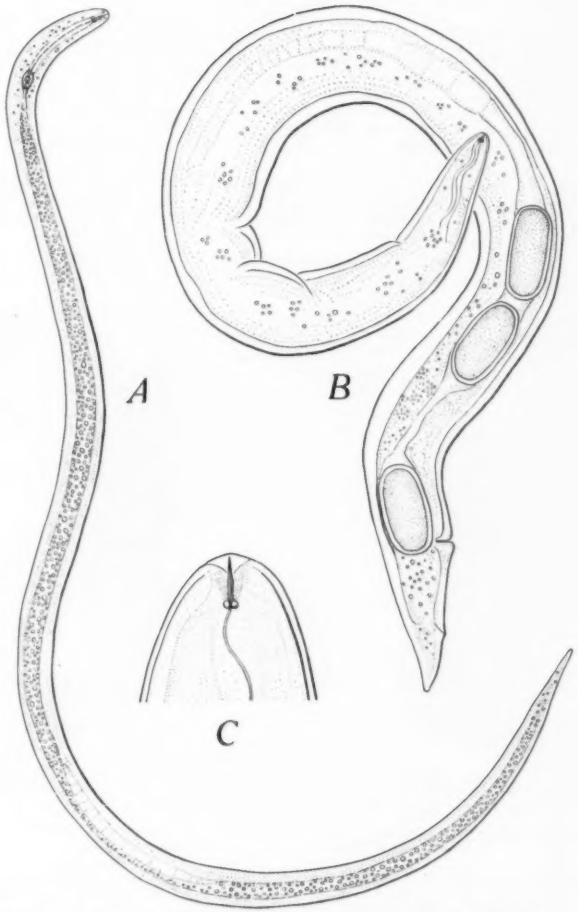

FIGURE 4.—*Anguillulina pustulicola*. *A*, Quiescent larva, × 320; *B*, female, dried and flattened, × 160; *C*, female head, × 800.

Relationship.—This species is most closely related to *Anguillulina agrostis* (Steinbach) Goodey, *A. graminis* (Hardy) Goodey, and *A. graminophila* Goodey. The principal morphological difference is that the lip region of *A. pustulicola* is continuous with the body contour, not set off as in the species mentioned.[8] *A. pustulicola* forms characteristic pustulelike galls upon grass stems, while *A. graminis* and *A. graminophila* inhabit only the leaves and *A. agrostis* the inflorescence.

ANGUILLULINA PHYLLOBIA, N. SP.

Leaves of *Solanum elaeagnifolium* Cav. infested with *A. phyllobia* were received from Harold R. Brisley, together with the following notes on the local occurrence and habits of the nema: [9]

Leaves of *Solanum elaeagnifolium* infested with nemas were first observed during the summer of 1922 near the town of Camp Verde, Ariz., but the injury was attributed to mites, and not until 1925 were nemas observed as the causal agents.

Solanum elaeagnifolium grows quite profusely in all parts of the Verde Valley. For 4 years close observation has been made here and in other areas of central and northern Arizona, but infestations have been found in only two areas, both within a mile of Camp Verde.

The field where infested plants were first discovered was a permanent pasture. In 1929 part of it was plowed and planted with corn, which later in the season

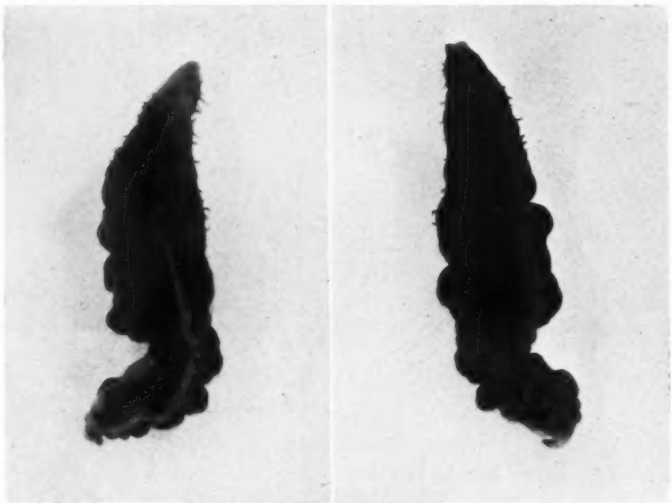

FIGURE 5.—Two views of *Solanum elaeagnifolium* leaf infested with *Anguillulina phyllobia*. Note unusual development of spines. Natural size.

harbored many weeds, including *Solanum elaeagnifolium*. However, the single plowing seemed to have eradicated the nemas, for none of the plants was found infested, while the unplowed portion exhibited the usual severe infestation.

Observations of definitely infested areas show that over a period of several years there apparently was no spread to adjacent, uninfested plants. The parasite shows a preference for plants growing in irrigated areas and along ditch banks which are constantly wet, or in areas where waste irrigation water frequently pools and the soil is uncultivated.

[8] GOODEY, T. THE GENUS ANGUILLULINA GERV. AND V. BEN., 1859, VEL TYLENCHUS BASTIAN, 1865. Jour Helminthology 10: 75–180. 1932.

[9] The first reports and specimens of this nema were received through the courtesy of Wyatt M. Jones and Jocelyn Tyler, Berkeley, Calif., who forwarded some of Brisley's material. Jones reported finding similar infestations in 1927 along the Rio Grande, above El Paso, Tex.

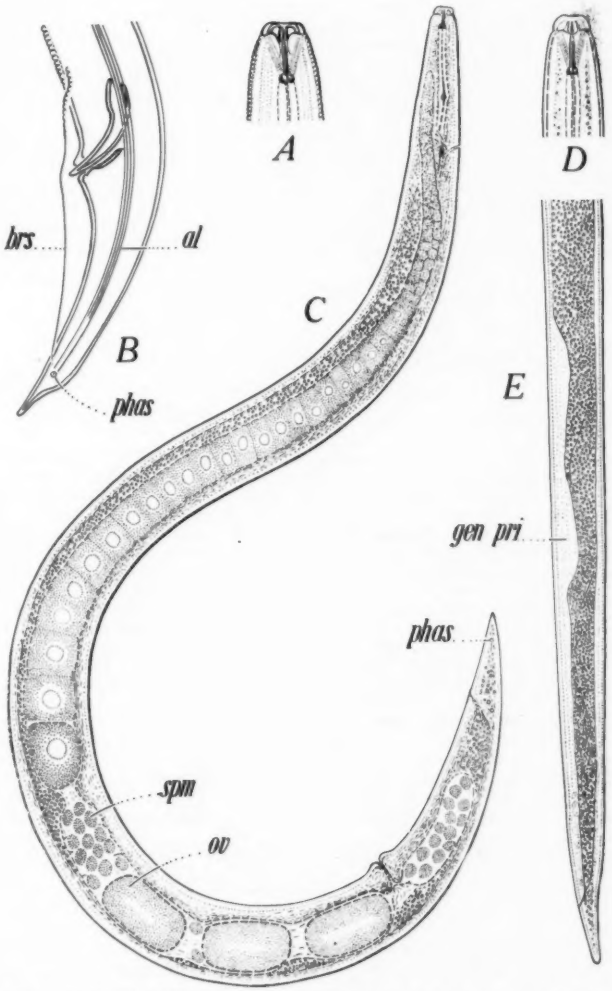

FIGURE 6.—*Anguillulina phyllobia*. *A*, Female head, × 800; *B*, male tail; *al*, wings; *brs*, bursa; *phas*, phasmid, × 800; *C*, female; *ov*, ovum; *phas*, phasmid; *spm*, spermatozoa, × 300; *D*, head of quiescent form, × 800; *E*, posterior portion of quiescent form: *gen pri*, genital primordium, × 200.

In this locality, *Solanum elaeagnifolium* begins to leaf out during the latter part of April and early May. Infestation first becomes apparent about the first of June, at which time the plant has made about 6 inches of growth. The first evidence of attack is a slight thickening and toughening of certain areas of the young leaves which have just unfolded. Soon after the young leaf is invaded, the presence of nemas can be determined by an unusual development of leaf spines where normally it would be difficult to see any spines at all. With the subsequent increase of infestation and development of galls, the spines become extremely numerous, stout, curved or deformed, and conspicuous reddish brown in color.

The leaf attains its full growth about 2 weeks after the first appearance of infestation, and by that time is hypertrophied almost beyond recognition. It appears to function in a nearly normal manner, and only after a period of 6 weeks or 2 months does it begin to show signs of necrosis (fig. 5).

If the leaf escapes infestation while in the bud, or at least when it is young and tender, it appears to be immune to the attack of the nemas. The first leaves to unfold in the spring show no infestation during the life of the plant.

Galled leaves may be found throughout the summer, and even as late as December when the plants are growing in locations protected from frost. During August new infestation appears to be at a minimum. A new period of infestation appears in the late summer and fall, probably the result of a second brood of nemas.

MORPHOLOGY

Measurements:

1.2	8.0	12.0	^{75}82.^{8}	94.5	0.75—0.95 mm
1.5	2.9	3.3	4.4	2.0	
1.1	9.2	13.5	^{85}M	94.5	0.70—0.85 mm
1.4	2.7	3.0	3.3	1.7	

General characters.—Transverse striae fine. Wing area marked by four bright refractive lines occupying a space equal to about one-tenth body width. Lip region four times as wide as high, definitely set off. Spear strongly knobbed, its length slightly greater than width of lip region. Deirids opposite base of esophagus. Medial bulb of esophagus much reduced; terminal bulb inconspicuous. Esophagus frequently pressed ventrad by ovary. Distance from vulva to anus equal to about twice tail length. Ovary outstretched, sometimes reaching medial bulb of esophagus. Posterior uterine branch frequently filled with spermatozoa, its length equal to about twice corresponding body diameter. Eggs slightly longer than body diameter at vulva, deposited before segmentation. Female tail uniformly conoid, acute. Male tail conoid, then somewhat digitate in posterior fifth. Bursa extending from a point opposite proximal ends of spicula to beginning of digitate portion of tail. Testis extending to medial bulb of esophagus, sometimes reflexed a short distance (fig. 6).

Quiescent form.—Average length, 0.62 mm; width, 25μ. Spear practically same size as that of adult, basal knobs slightly less developed. Genital primordium translucent, no differentiation between sexes. Tail conical, terminus slightly rounded. Dried leaves contain thousands of these immature specimens, which pass through these unfavorable conditions in a dormant stage, and revive immediately upon being moistened. Estimated population in leaf (fig. 5), 842,000.

Diagnosis.—*Anguillulina* with above measurements. Area between wings about one-tenth body width. Lip region about four times as wide as high, definitely set off. Ovary extending past esophagus base. Posterior uterine branch about twice as long as body width. Distance from vulva to anus equal to about twice tail length. Male tail conoid, then digitate in posterior fifth. Bursa rising about opposite proximal ends of spicula, extending to digitate portion of tail. Causal agent of hypertrophied leaves of *Solanum elaeagnifolium*.

NEOTYLENCHUS OBESUS, N. SP.

An alfalfa crown collected near Greeley, Colo., bore small pitlike lesions from which eight females of *Neotylenchus obesus* (fig. 7) were taken. These lesions, of which the nemas appeared to be the causal agent, each contained from 1 to 3 individuals. Only 1 infested

crown was found among several examined, and subsequent collections in the same and neighboring fields failed to reveal additional specimens. Therefore, the species appears to be very limited in its distribution.

MORPHOLOGY

Measurements.—Length, 0.7 to 0.9 mm; width, 55μ to 70μ; spear length, 7μ; vulva, from 95 percent to almost terminal.

General characters.—Body obese, somewhat cylindrical, variable in form and width. Striae very fine. Wings not visible but doubtless present as in other species of the genus. Lip region continuous with neck contour, the lips low and obscure. A face view shows the eight cephalic sectors typical of the genus (fig. 7, *C*). Labial framework obscure. Spear short and slender with three well-defined basal knobs. Esophagus: corpus somewhat spindle-shaped; isthmus narrow, bulb elongate but pressed out of shape by ovary. Nerve ring encircling isthmus. Excretory pore about opposite nerve ring. Intestine densely granular. Rectum and anus invisible, probably absent. Vulva a broad, elevated, transverse slit. Vagina extending half way across body at almost right angles to body axis. Uterus single, ovary outstretched, sometimes

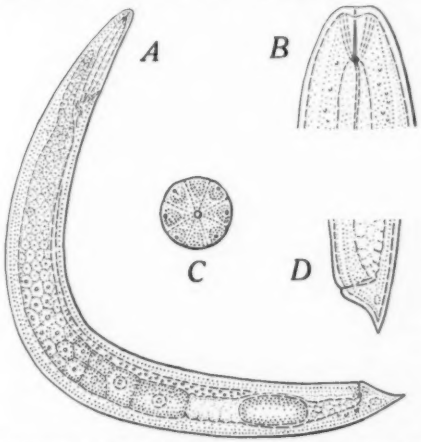

FIGURE 7.—*Neotylenchus obesus*. *A*, Female, × 150; *B*, head, × 1,000; *C*, face view, × 1,500; *D*, posterior portion of female with short tail, × 250.

reaching to spear. Eggs, 22μ (19μ–25μ) by 53μ (52μ–58μ), as many as five observed in the uterus at one time. Male unknown. Sperms not observed in gravid females.

Diagnosis.—Obese *Neotylenchus*. Labial framework obscure. Spear short and slender. Ovary large, reaching past esophagus base. Vulva, from 95 percent to almost terminal. Rectum and anus apparently absent. Probable causal agent of small lesions on alfalfa crowns.

SUMMARY

The nematode *Anguillulina pratensis* is the causal agent of a destructive root disease of figs in California. Symptoms and known distribution of this disease are here discussed.

Morphological descriptions and disease symptoms produced are given for three new plant-parasitic nematodes: *Anguillulina pustulicola*, parasitic on stems of an unidentified grass; *A. phyllobia*, infesting *Solanum elaeagnifolium;* and *Neotylenchus obesus*, found inhabiting lesions on alfalfa crowns.

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